Peptides 54 (2014) 49–57

Contents lists available at ScienceDirect

Peptides

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

Plasma somatostatin-like immunoreactivity increases in the plasma of septic patients and rats with systemic inflammatory reaction: Experimental evidence for its sensory origin and protective role

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ARTICLE INFO

Article history:
Received 11 September 2013
Received in revised form 12 January 2014
Accepted 13 January 2014
Available online 20 January 2014

Keywords:
Adhesion molecules
Capsaicin-sensitive sensory nerves
Coagulation factors
Cecum puncture model
Cytokines
Radioimmunoassay
Somatostatin
Cyclo-somatostatin
Systemic inflammatory response syndrome

ABSTRACT

Alterations of somatostatin-like immunoreactivity (SST-LI) in the plasma of 11 systemic inflammatory response syndrome (SIRS) patients were investigated in correlation with cytokines, adhesion molecules and coagulation markers repeatedly during 4 days. The origin and role of SST were studied in the cecum ligation and puncture (CLP) rat SIRS model. Capsaicin-sensitive peptidergic sensory nerves were defunctionalized by resiniferatoxin (RTX) pretreatment 2 weeks earlier, in a separate group animals were treated with the somatostatin receptor antagonist cyclo-somatostatin (C-SOM). Plasma SST-LI significantly elevated in septic patients compared to healthy volunteers during the whole 4-day period. Significantly decreased Horowitz score showed severe lung injury, increased plasma C-reactive protein and procalcitonin confirmed SIRS. Soluble P-selectin, tissue plasminogen activator and the interleukin 8 and monocyte chemotactic protein-1 significantly increased, interleukin 6 and soluble CD40 ligand did not change, and soluble Vascular Adhesion Molecule-1 decreased. SST-LI significantly increased in rats both in the plasma and the lung 6 h after CLP compared to sham-operation. After RTX pretreatment SST-LI was not altered in intact animals, but the SIRS-induced elevation was absent. Lung MPO activity significantly increased 6 h following CLP compared to sham operation, which was significantly higher both after RTX-desensitization and C-SOM-treatment. Most non-pretreated operated rats survived the 6 h, but 60% of the RTX-pretreated ones died showing a significantly worse survival. This is the first comprehensive study in humans and animal experiments providing evidence that SST is released from the activated peptidergic sensory nerves. It gets into the bloodstream and mediates a potent endogenous protective mechanism.

1. Introduction

Sepsis is a severe systemic inflammatory condition which is often lethal even despite the available combined antibiotic treatment and intensive therapy [2,56]. Therefore, there is a great need for precise understanding its complex pathophysiological background and identifying novel therapeutical targets [16]. The nervous, endocrine and immune systems have strong interactions in these processes, neuro-immune and neuro-endocrine cross-talks play an important role in these inflammatory conditions affecting several organ systems [14]. Neuropeptides, adhesion and coagulation factors, as well as cytokines are the most important regulators of these mechanisms. Neuropeptides released from peripheral terminals of sensory neurons in response to a variety of stimuli generated upon tissue damage exert nociceptive or anti-nociceptive, as well as pro-inflammatory or anti-inflammatory actions. Besides the pro-inflammatory peptides like substance P or calcitonin gene-related peptide, somatostatin (SST) is also released from the same nerve endings, neuroendocrine, inflammatory and immune cells in response to a wide range of inflammatory mediators [36]. We have provided evidence in animal models that SST released from the activated sensory nerve endings exerts not

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0196-9781/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.peptides.2014.01.006

only a local interaction with inflammatory mediators, but it gets into the systemic circulation and mediates anti-inflammatory and anti-nociceptive "sensocrine" actions at distant parts of the body [51–53]. Furthermore, our animal experimental results described several lines of evidence in a variety of mechanism and disease models that somatostatin has very potent anti-inflammatory and analgesic actions acting on vessels, inflammatory cells and sensory nerve terminals [21,39,48,51,52]. These are mainly mediated by the somatostatin sst_4 receptor, which is one of the 5 cloned G_i protein-coupled receptors of this peptide. The inhibition of the production of several hormones (e.g. insulin, prolactin, growth hormone) and other transmitters like serotonin is mediated by the sst_2, sst_3 and sst_5 receptors, but sst_4 stimulation does not have any endocrine actions [37].

We have earlier provided the first human data that plasma somatostatin (SST) level increases during and after abdominal [3], thoracic and orthopedic surgery, as well as showed in an exploratory study of 11 patients that there is an SST elevation also in sepsis [50].

The widely used sensitive sepsis markers in the clinical practice to diagnose the infection and monitor the therapeutic response are plasma C-reactive protein (CRP) and procalcitonin (PCT). CRP is an acute-phase protein, which has a central role in immunological processes in sepsis. It is synthesized in the liver mainly in response to IL-6 and binds to polysaccharides of pathogens promoting phagocytosis [28]. PCT is a glycoprotein having a very long, 25–30 h plasma elimination half-life. In normal circumstances it is produced in the C cells of the thyroid gland. The source of PCT is not identified in sepsis, but it is presumably produced by leukocytes, neuroendocrine cells and lung epithelial cells. PCT has an important role in identifying sepsis [11,30].

Other plasma factors that might be of interest in septic patients as markers for diagnosis, following the effectiveness of the therapy, or finding novel therapeutic approaches are cytokines and adhesion molecules. The CD40 ligand (sCD40L) is soluble and stored in platelet granules and it is a marker of platelet activation. The interaction between CD40 localized on the surface of smooth muscles and endothelial cells and sCD40L is triggering the release of inflammatory mediators, which activates the coagulation cascade by increasing the activity of metalloproteinases involved in inflammatory reactions [27]. Tissue plasminogen activator (tPA) is one of the proteins responsible for the breakdown of blood clots in the vasculature. It is routinely measured for the diagnosis of thrombotic, embolic and hemorrhagic abnormalities [42]. The Monocyte Chemotactic Protein-1 (MCP-1) is an inducible inflammatory cytokine responsible for the recruitment of macrophages, memory T and dendritic cells to the location of inflammation, infection and injury [45]. Plasma soluble P-selectin' (sP-selectin) and Vascular Cell Adhesion Molecule-1 (VCAM-1) regulate inflammatory cell migration and extravasation, their levels have been shown to increase in several inflammatory conditions [31]. Interleukin 6 (IL-6) is an important and interesting cytokine with both pro- and anti-inflammatory properties. Its level is increased in the blood due to inflammation/infection, tissue damage, trauma, burns and sepsis [20]. IL-8 is a major chemotactic factor belonging to the CXC chemokine group and a major mediator of inflammation. This is released from several cells and has got a very potent angiogenic feature. The main role of this molecule is the induction of chemotaxis in leucocytes. By increasing intracellular Ca^{2+} level, exocytosis or respiratory burst its main function is phagocytosis and migration which is the result of very complicated series of physiological reactions is also one of the main functions of neutrophils [22].

Since our previous preliminary study showed an elevation of plasma SST levels in sepsis, the aim of the present work was to analyze the time-dependent alterations of SST-like immunoreactivity (SST-LI) in further patients to determine if SST release into the systemic circulation is a general response to a severe systemic inflammatory reaction. The correlations of SST-LI with other established sepsis markers and interesting, potentially useful laboratory parameters, as well adhesion molecules and cytokines described above were simultaneously investigated. Furthermore, a widely used rat model of severe systemic inflammation was also performed to identify the source and role of the released SST.

2. Materials and methods

2.1. Human studies: protocol and analytical methods

2.1.1. Patients

Eleven septic patients (6 males and 5 females; mean age of 64 ± 8.12 years, males 60 ± 6.69 years, females 67 ± 2.82 years) were enrolled in the study. All septic patients admitted to Intensive Care Unit were continuously investigated on a daily basis. The human studies were approved by the Ethics Committee of University of Pécs (3362). The patients or in cases of their inabilities their relatives signed a written informed consent prior to the participation. The definition and criteria to determine the Systemic Inflammatory Response Syndrome (SIRS), sepsis or septic shock were used according to the Consensus Conference of the American College of Chest Physicians/Society of Critical Care Medicine in 1992 [6].

Clinical signs of sepsis were the following: SIRS (American College of Chest Physicians and Society of Critical Care Medicine, 1990 Consensus Conference: temperature >38.5 °C or <36.5 °C, heart rate > 90/min, respiratory rate > 20/min, 4000 > WCC > 12,000 or ratio of immature forms > 10%) plus presumed or confirmed infectious process. The source (or suspected source) of infection was cultured regularly. Every patient was treated the same way according to the Surviving Sepsis Campaign: International guidelines for management of severe sepsis and septic shock 2008 and practice pattern did not change during the study period [15].

2.1.2. Analytical methods

The Horowitz index (score) was also determined routinely to follow the severity of lung damage and confirm the presence of sepsis and/or SIRS. It is defined as the ratio of arterial oxygen partial pressure (paO_2, arterial blood gas analysis) and the concentration of oxygen in the inhaled air (FiO_2, inspiratory oxygen concentration). In healthy lungs the Horowitz score depends on the age and the value is between 350 and 450. A value below 300 refers to a moderate acute lung injury, and a value below 200 is a criteria of a severe lung injury (acute respiratory distress syndrome: ARDS) [41].

All patients were either fasted or had parental nutrition only and were treated with broad spectrum antibiotics or a certain type of antibiotics according to the sensitivity spectrum of the identified bacteria. Patients were intubated, sedated with the mixture of propofol (Propofol 2%, Fresenius Kabi Deutschland, Germany) and morphine 1 mg/ml, 0–4 ml/h (TEVA Hungary), as the clinician decided and mechanically ventilated (CPAP, PEEP/FiO_2 as required, PS: 6–8 ml/kg) in order to improve or maintain oxygenation. For hemodynamical stability patients were given vasopressor or inotropic infusion of noradrenaline or adrenaline 3–9 mg/50 ml (Richter Gedeon Hungary), dobutamine (250 mg/50 ml, Aspen Pharmacare Australia Pty Ltd.) as clinical picture required and urine output were maintained.

The first blood samples for the laboratory analysis were taken on the first day of the admission (day 1) and then repeated every day at 8.00 o'clock in the morning for 4 days. The 2×5 ml of blood samples were immediately taken into ice-cold EDTA-containing Vacutainers (18 mg REF 367525 and 143 I.U. REF 367674) to prevent clotting,