Peptides 47 (2013) 133–141

Contents lists available at ScienceDirect

Peptides

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

Effects of corticotropin-releasing hormone on proopiomelanocortin derivatives and monocytic HLA-DR expression in patients with septic shock

Reginald Matejec[a,b,*], Friederike Kayser[b], Frauke Schmal[a], Florian Uhle[a], Rolf-Hasso Bödeker[c], Hagen Maxeiner[a], Julia Anna Kolbe[a]

[a] Department of Anaesthesiology, Intensive Care Medicine and Pain Therapy, Justus-Liebig-University, Rudolf-Buchheim-Str. 7, D-35392 Giessen, Germany
[b] Rudolf-Buchheim-Institute of Pharmacology, Justus-Liebig-University, Frankfurter Str. 107, D-35392 Giessen, Germany
[c] Institute of Medical Statistics and Informatics, Justus-Liebig-University, Heinrich-Buff-Ring 44, D-35392 Giessen, Germany

ARTICLE INFO

Article history:
Received 25 April 2013
Received in revised form 9 July 2013
Accepted 9 July 2013
Available online 25 July 2013

Keywords:
Proopiomelanocortin (POMC)
Septic shock
Corticotropin releasing hormone (CRH)
Alpha melanocyte stimulating hormone (α-MSH)
Human leukocyte antigen-DR (HLA-DR)
Tumor necrosis factor-α (TNF-α)

ABSTRACT

Little is known about interactions between immune and neuro-endocrine systems in patients with septic shock. We therefore evaluated whether the corticotropin-releasing hormone (CRH) and/or proopiomelanocortin (POMC) derivatives [ACTH, β-endorphin (β-END), β-lipotropin (β-LPH), α-melanocyte stimulating hormone (α-MSH) or N-acetyl-β-END (Nac-β-END)] have any influences on monocyte deactivation as a major factor of immunosuppression under septic shock conditions. Sixteen patients with septic shock were enrolled in a double-blind, cross-over and placebo controlled clinical study; 0.5 μg/($kg_{bodyweight}$ h) CRH (or placebo) were intravenously administered for 24 h. Using flow cytometry we investigated the immunosuppression in patients as far as related to the loss of leukocyte surface antigen-DR expression on circulating monocytes (mHLA-DR). ACTH, β-END immunoreacive material (IRM), β-LPH IRM, α-MSH and Nac-β-END IRM as well as TNF-α and mHLA-DR expression were determined before, during and after treatment with CRH (or placebo). A significant correlation between plasma concentration of α-MSH and mHLA-DR expression and an inverse correlation between mHLA-DR expression and TNF-α plasma level were found. Additionally, a significant increase of mHLA-DR expression was observed 16 h after starting the CRH infusion; 8 h later, the mHLA-DR expression had decreased again. Our results indicate that the up-regulation of mHLA-DR expression after CRH infusion is not dependent on the release of POMC derivatives. From the correlation between plasma concentration of α-MSH and mHLA-DR expression, we conclude that in patients with septic shock the down-regulation of mHAL-DR expression is accompanied by the loss of monocytic release of α-MSH into the cardiovascular compartment.

1. Introduction

In patients with septic shock the deactivation of monocytes has been identified as a major factor of immunosuppression and is associated with a loss of surface human leukocyte antigen-DR expression on circulating monocytes. This is supported by several studies [3,11,29] demonstrating a link between the loss of HLA-DR expression on monocytes (mHLA-DR) and impairment of monocytic cellular functions, including their loss of pro-inflammatory properties, e.g. a failure in antigen presentation [11] or their reduced release of pro-inflammatory cytokines such as IL-1 and TNF-α into the cardiovascular compartment [3].

Furthermore, an activation of the hypothalamic–pituitary–adrenal (HPA) axis by release of corticotropin releasing hormone (CRH) was observed in patients with septic shock [5]. In turn, CRH stimulates the anterior pituitary gland to release corticotroph-type (ACTH, β-endorphin immunoreactive material (β-END IRM), β-lipotropin (β-LPH) IRM) and melanotroph-type (N-acetyl-β-END IRM, α-MSH) proopiomelanocortin (POMC) derivatives into the cardiovascular compartment [16].

So far, little is known about interactions between immune and neuro-endocrine systems in patients under septic shock. However, α-MSH, a melanotroph-type POMC-derived peptide has potent anti-inflammatory effects in animal [10] and human [7] models of acute or chronic inflammation. In particular, it was shown in in vitro studies that α-MSH reduces the release of lipopolysaccharide (LPS)-elicited production of tumor necrosis factor (TNF)-α and interleukin (IL)-1β [7]. Furthermore, it was observed that α-MSH inhibited LPS-induced proteolytic enzyme release, oxidative burst

* Corresponding author at: Justus-Liebig-University of Giessen, Department of Anaesthesiology, Intensive Care Medicine and Pain Therapy, Rudolf-Buchheim-Str. 7, D-35392 Giessen, Germany. Tel.: +49 641 99 44402; fax: +49 641 99 44409.
E-mail address: reginald.matejec@chiru.med.uni-giessen.de (R. Matejec).

0196-9781/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.peptides.2013.07.013

response, nitric-oxide (NO) production, and reactive oxygene intermediate generation in monocytes [22]. Additionally, in vitro it was shown that α-MSH reduced the endotoxin receptor CD14 expression on the surface of macrophages, and it appears that α-MSH exerts part of its anti-inflammatory effect through a reduction of CD14 [22]. It is currently expected that the melanocortin system in leukocytes contributes to protecting the host from injury caused by excessive host reactions [7]. It was recently shown that after injecting CRH the net areas under the concentration curves of α-MSH were significantly lower in nonsurvivors than in survivors in patients with septic shock [16]. Based on these in vivo results it is thus likely that α-MSH is a part of the host defense to limit the inflammatory response of the immune system in patients under septic shock.

As yet, there is no clinical study which has explored influences of α-MSH on mHLA-DR expression in patients with septic shock. However, direct tests of cause and effect (i.e. an intravenous administration of α-MSH in patients) during septic shock are not possible for ethical reasons. Thus, in 16 patients with septic shock, but without adrenal insufficiency, we studied the response of the melanotroph- and the corticotroph-type POMC system to intravenous (i.v.) administration of 0.5 μg/$kg_{bodyweight}$ CRH over a period of 24 h. In addition, the mHLA-DR expression and TNF-α were monitored simultaneously. This experimental design allowed us to explore the effects of CRH-administration (and the subsequent α-MSH release into the cardiovascular compartment) on levels of TNF-α and mHLA-DR expression in patients under the conditions of septic shock.

2. Materials and methods

2.1. Patients

This prospective study in patients was performed in an intensive care unit (ICU) of a university hospital. At the onset of severe sepsis, patients of the ICU were subjected to an ACTH stimulation test using an i.v. bolus of 250 μg tetracosactin (Synacthene®, Novartis Pharma, Nürnberg, Germany). The ACTH stimulation tests were performed between 8:00 h (8:00 AM) and 16:00 h (4:00 PM). Blood samples were taken immediately before, 30 and 60 min after the test. Cortisol response was defined as the difference between the highest of the concentrations taken after the test and those taken before the test. Adrenocortical insufficiency was defined by a response concentration of less than 248 nmol/L (9 μg/dL) [1]. Non-responders to the ACTH stimulation test were excluded from the study; only those responders were included who met the criteria of septic shock proposed by the American College of Chest Physicians and the Society of Critical Care Medicine Consensus Conference Committee of 1992 [30]. Approval of the study was obtained from the Ethics Committee of the University of Giessen (Medical Faculty), written consent to participate in the study was given by 16 patients or their surrogates (characteristics see Table 1). Exclusion criteria were an age less than 18 years, pregnancy, known endocrine disorders, immunosuppressive medication, chemotherapy, radiation therapy or cardiopulmonary resuscitation within one month before the study and life expectancy of less than two months as well as administration of corticosteroids, opiates or etomidate within 48 h before study commencement.

2.2. Study protocol

At the onset of septic shock, we studied in 16 patients the effect of intravenously administered CRH (0.5 μg/($kg_{bodyweight}$ h)) over a period of 24 h on the release of POMC derivatives such as ACTH, β-END IRM, β-LPH IRM, α-MSH, and Nac-β-END, as well as their influences on mHLA-DR expression during septic shock.

The study utilized a cross-over design with a double-blind control. All patients were assigned randomly to take part in two groups: group A: patients initially received CRH (0.5 μg/($kg_{bodyweight}$ h) over a period of 24 h) via infusion-pump, afterwards the syringe was changed and patients immediately received placebo (saline 0.9%) (also over a period of 24 h) and group B: patients initially received placebo (saline 0.9%) over a period of 24 h via infusion pump, subsequently the syringe was changed and patients received CRH (0.5 μg/($kg_{bodyweight}$ h)) over a period of 24 h.

To evaluate the indirect influences of CRH on mHLA-DR expression via the release of POMC derivatives, we determined plasma concentrations of ACTH, β-END IRM, β-LPH IRM, α-MSH, and Nac-β-END IRM as well as the expression of HLA-DR on circulating monocytes by flow cytometry at six different times: before starting the CRH (group A) or the placebo (saline 0.9%) (group B) infusion-pump (time t_0), 16 (time t_1), and 24 h (time t_2) after starting the treatment with CRH (or placebo) via infusion-pump, 16 (time t_3) and 24 h (time t_4) after changing syringes for starting the second treatment, as well as 16 h after having finished the second treatment (placebo or CRH) at time t_5 (see Fig. 1). In addition, sepsis-related cytokines (TNF-α) were determined all six times.

All the infusions started at the same time of day (around 16:00 h (4:00 PM)); the first blood samples were taken at time t_0 and the infusion of the first syringe (CRH in case of group A, saline 0.9% in case of group B) was started. On the following day blood samples were taken at t_1 (around 8:00 h (8:00 AM)) and t_2 (around 16:00 h (4:00 PM)); immediately after t_2 the syringe was changed and the patients received saline 0.9% (group A) or CRH (group B). On the

Table 1
General characteristics of the 16 patients with septic shock enrolled in the study (BMI: body mass index; ARDS: acute respiratory distress syndrome; COPD: chronic obstructive pulmonal disease).

Patient no.	Age (yrs)	BMI (kg/m^2)	Gender	Diagnosis/infections	Isolated microorganism	Antibiotics, concomitant treatments
1	67	35.3	Female	Necrotizing pancreatitis	*Enterococcus faec., Cand. albic.*	Imipenem, vancomycin, selen
2	46	25.8	Male	COPD, stomach perforation	*Enterobacteriaceae*	Imipenem, ciprofloxacin, selen
3	71	34.9	Male	Peritonitis, COPD, pneumonia	*Staph. aureus, Candida albicans*	Vancomycin, fluconazol, selen
4	55	27.5	Male	ARDS, pneumonia	*Staphylococcus aureus*	Vancomycin, tobramycin, selen
5	60	26.8	Female	Stomach bleeding, pneumonia	*Enterobacteriaceae*	Imipenem, metronidazol, selen
6	45	24.2	Male	Multiple trauma, pneumonia	*Staph. aureus; Enteroco. faec.*	Imipenem, vancomycin, gentamycin, selen
7	68	29.8	Male	Peritonitis, pneumonia	*Enterobacteriaceae*	Imipenem, metronidazol, selen
8	68	26.3	Male	Necrotizing pancreatitis	*Staph. aureus, Candida glabrata*	Imipenem, Vancomycin, flucytosin, selen
9	72	34.7	Male	Acute necrotizing fasziitis	*Staphylococcus aureus, E. coli*	Imipenem, vancomycin, selen
10	67	28.5	Male	Peritonitis, pneumonia	*Pseudom. aerugin.; Staph. aureus*	Imipenem, vancomycin, selen
11	69	29.4	Male	Peritonitis, ARDS	*Enterococcus faec., Serrat. marc.*	Vancomycin, gentamycin, selen
12	48	34.1	Male	Stomach bleeding, pneumonia	*Staphylococcus aureus*	Vancomycin, tobramycin, selen
13	67	38.2	Female	Colon cancer, COPD	*Staphylococcus aureus*	Vancomycin, metronidazol, selen
14	62	32.9	Female	Multiple trauma, pneumonia	*Staphylococcus aureus*	Vancomycin, imipenem, selen
15	47	22.6	Female	Stomach cancer, pneumonia	*Candida glabrata*, peptococcus	Imipenem, fluconacol, clindamycin selen
16	62	33.8	Female	Peritonitis, pneumonia	*Enterococcus faecalis*	Imipenem, metronidazol, selen