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BRIEF REPORT

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The influence of corticosteroids on the release of novel biomarkers in human endotoxemia

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Abstract *Objective:* Sepsis intervention studies need better patient stratification methods, and one way to realize this is the introduction of stable biomarkers. A set of recently developed novel biomarkers, based upon precursor-fragments of short-lived hormones, was previously shown to be increased during sepsis. However, it is not known whether these biomarkers are influenced by sepsis intervention strategies. Therefore we investigated the markers in a model of human endotoxemia intervened by increasing doses of prednisolone. *Design and setting:* Prospective, open-label study in a specialized clinical research unit of a university hospital. *Subjects:* Thirty-two healthy male volunteers. *Interventions:* Subjects received prednisolone orally at doses of 0, 3, 10 or 30 mg ($n = 8$ per group) at 2 h before intravenous injection of *Escherichia coli* lipopolysaccharide (LPS) (4 ng/kg). Blood samples were drawn during 24 h after LPS injection. *Measurements and results:* LPS injection caused an increase in levels of midregional pro-adrenomedullin (MR-proADM), midregional pro-atrial natriuretic peptide (MR-proANP), C-terminal pro-arginine–vasopressin (CT-proAVP) and procalcitonin (PCT). Prednisolone caused a dose dependent inhibition of MR-proADM, MR-proANP and CT-proAVP levels. *Conclusions:* These results show that a set of novel, highly stable sepsis biomarkers was increased during human endotoxemia and was dose-dependently inhibited by corticosteroid pre-treatment.

Keywords Corticosteroids · Biological markers · Endotoxin · Sepsis

Introduction

During the past decade numerous sepsis intervention strategies have been introduced; only few of them, however, have shown beneficial effects. Major problems in sepsis intervention trials were mixed results and unclear risk stratification methods. The lack of clear stratification methods was especially disturbing, since the effectiveness of intervention in these studies correlated closely with disease severity and risk of mortality [1]. Within this context, there is a need for biomarkers to predict disease severity and mortality risk.

During sepsis a wide range of inflammatory proteins is released and in theory all could be used as a biomarker. The vast majority of these proteins is biologically active though and as a consequence rapidly cleared from the circulation. A promising new approach is the measurement of stable prohormone fragments of bioactive peptide-hormones [2–4]. In theory, these fragments often have no known biological function during sepsis and are therefore less rapidly cleared and less sensitive to small homeostatic changes [5–7].

The most prominent prohormone sepsis marker is procalcitonin (PCT), which represents the precursor hormone of calcitonin. Its diagnostic and prognostic properties were superior to C-reactive protein (CRP) in multiple studies [8]. In addition, PCT responded to sepsis intervention strategies and represented a promising marker for monitoring of treatment effect [9, 10]. In recent years, additional assays have been developed for measurement of stable prohormone fragments, such as mid-regional pro-adrenomedullin (MR-proADM) [5], derived from the precursor of adrenomedullin (ADM); mid-regional pro-atrial natriuretic peptide (MR-proANP), derived from the precursor of atrial natriuretic peptide (ANP) [6]; and C-terminal proAVP (CT-proAVP), derived from the precursor of arginine–vasopressin (AVP; also known as the antidiuretic hormone) [7]. Levels of these biomarkers were higher in samples from patients with sepsis [2–4]. Yet it is not known whether these levels are influenced by a potential therapeutic sepsis intervention. In order to investigate the response of the novel biomarkers to a sepsis intervention strategy we applied the model of human endotoxemia and pre-treated subjects by increasing doses of corticosteroids, which is one of the few relatively successful, and among the most cost-effective, septic shock intervention strategies to date [11].

Methods

Subjects and study design

The study was designed according to the requirements of the Declaration of Helsinki and approved by the institutional scientific and ethics committees, and written informed consent was obtained from all subjects. The study was performed simultaneously with an investigation of the effects of prednisolone on activation of the cytokine network, coagulation and hemodynamic variables in the same cohort [12]. Thirty-two healthy male volunteers [mean (± SE) age 23.9 ± 0.7 years] were admitted to a hospital clinical research unit. Screening tests were all normal. Prednisolone (prednisolone sodium phosphate oral solution 5 mg/ml; prepared by institutional pharmacy) was administered orally at a dose of 0, 3, 10 or 30 mg ($n = 8$ per group) at 2 h prior to LPS injection. All participants were challenged at $t = 0$ h with LPS (Escherichia coli lipopolysaccharide, lot G; US Pharmacopeia, Rockville, MD) as a bolus intravenous injection at a dose of 4 ng/kg, which is a standardized dose [13].

Assays

Blood was collected at intervals from 2 h before LPS injection to 24 h thereafter. Blood was immediately centrifuged (4 °C, 10 min, 3000 rpm) and plasma was stored at −20 °C until assayed. Uptake of orally administered prednisolone was measured by HPLC-MS/MS technology (CombinatoRx, Inc., Boston, MA, USA). MR-proADM, MR-proANP, CT-proAVP and PCT were measured by chemiluminescence label-coated tube-based sandwich immunoassays (Brahms Diagnostica, Berlin, Germany) as previously described [5–7, 14].

Statistical analysis

Differences between treatment groups were analyzed using a non-parametric mixed model approach (repeated-measures ANOVA). The effects reported are treatment effects adjusted for time effects. p values < 0.05 were considered statistically significant. Values presented are given as mean ± SE.

Results

Pharmacokinetic data

As shown in Fig. 1, uptake of orally administered prednisolone was demonstrated by a dose-dependent increase in plasma levels during endotoxemia. The levels peaked from 1 h before LPS administration to 1 h after LPS administration (peak levels 302 ± 34 ng/ml, 190 ± 19 ng/ml and 71 ± 5 ng/ml in the groups receiving prednisolone 30, 10 and 3 mg respectively; $t = -1$ h).

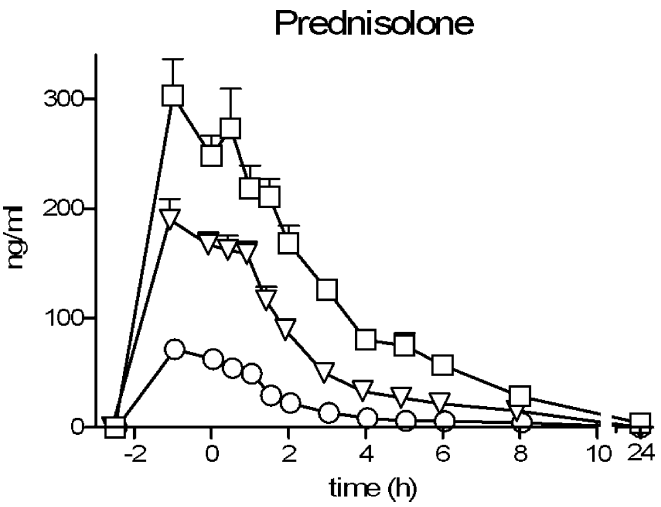

Fig. 1 Prednisolone. Mean (± SE) values of prednisolone concentrations after LPS administration (4 ng/kg IV, $t = 0$ h) to healthy male volunteers, preceded by oral administration of prednisolone 3 mg (*circles*), 10 mg (*triangles*), or 30 mg (*squares*) at $t = -2.5$ h

pre-treatment (levels at $t = 4$ h: 0.65 ± 0.06 nmol/l and 0.65 ± 0.1 nmol/l respectively; $p < 0.05$), but not after the low dose of prednisolone 3 mg. MR-proANP levels rose from 42.4 ± 2.2 pmol/l at baseline to 118.4 ± 17.2 pmol/l in the control group at $t = 4$ h. These levels were reduced after prednisolone 30 mg and 10 mg (peak levels: 91.5 ± 9.9 pmol/l and 64.8 ± 5.6 pmol/l at $t = 2$ h and $t = 4$ h, respectively; $p < 0.05$). CT-proAVP levels showed a modest increase from 12.5 ± 4.0 pmol/l to 18.0 ± 4.8 pmol/l in the control group at $t = 4$ h. The rise was inhibited after prednisolone 30 mg (peak level: 6.1 ± 1.0 pmol/l; $p < 0.05$) and not significantly changed by 10 mg (peak level: 10.6 ± 3.4 pmol/l; $p < 0.1$). In contrast, prednisolone 3 mg pre-treatment was associated with enhanced CT-proAVP release (peak level 34.7 ± 11.5 pmol/l; $p < 0.05$). PCT levels increased from a baseline level of 0.04 ± 0.02 ng/ml to a peak value of 16.7 ± 2.6 ng/ml in the control group. Peak PCT levels all occurred at $t = 24$ h and were not significantly affected by prednisolone pre-treatment (11.9 ± 3.0 ng/ml, 13.1 ± 2.5 ng/ml and 17.6 ± 3.5 ng/ml in the 30, 10 and 3 mg group, respectively).

Novel prohormone biomarkers

MR-proADM levels increased after LPS administration from 0.31 ± 0.02 nmol/l at baseline to a peak value of 1.05 ± 0.11 nmol/l in the control group at $t = 4$ h (Fig. 2). The rise was inhibited after prednisolone 30 mg and 10 mg

Discussion

The primary objective of this study was to determine the effect of increasing doses of prednisolone on the release of novel biomarkers during human endotoxemia. We show

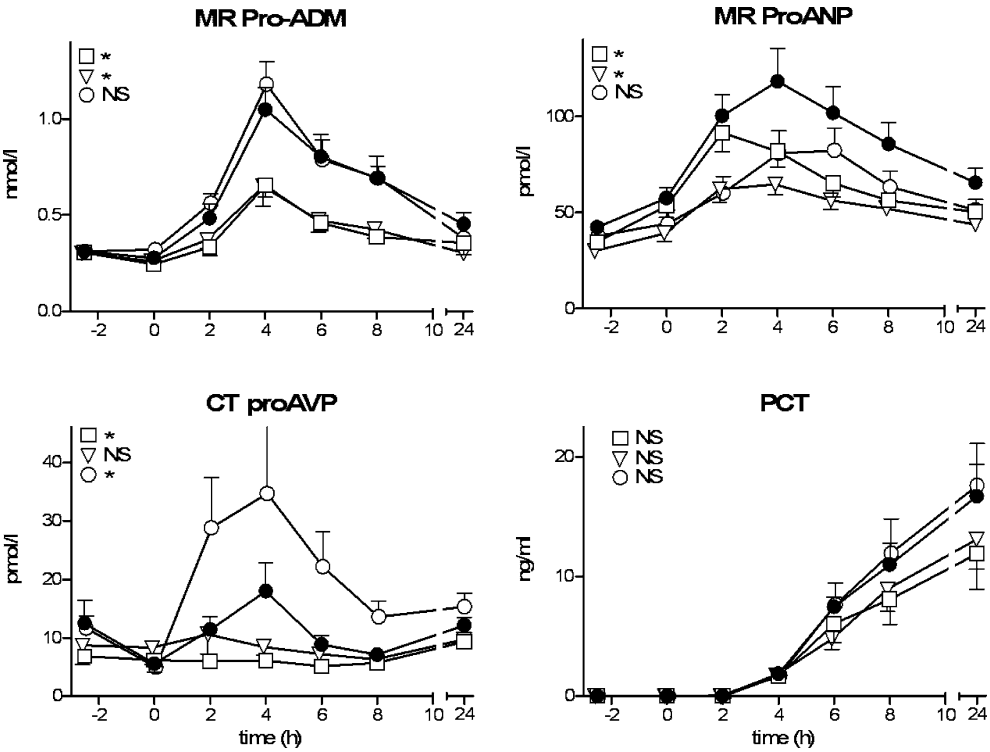

Fig. 2 Novel prohormone biomarkers. Mean (± SE) values of MR-proADM, MR-proANP, CT-proAVP and PCT after LPS administration (4 ng/kg IV, $t = 0$ h) to healthy male volunteers, preceded by oral administration of prednisolone 3 mg (*open circles*), 10 mg (*triangles*), 30 mg (*squares*) or, in the control group, 0 mg (*filled circles*) at $t = -2.5$ h. * $p < 0.05$ vs. control group. *NS*, non significant

here that prednisolone pharmacologic concentrations peaked at the time of LPS infusion and that the release of MR-proADM, MR-proANP and CT-proAVP was subsequently inhibited in a dose-dependent way. The levels of PCT were not significantly inhibited within the study period of 24 h. However, previously inhibition of PCT was shown by anti-inflammatory agents within a period of 168 h [9]. The levels of CT-proAVP were, remarkably, increased after prednisolone 3 mg, yet the reason for this remains unclear because the pattern was unique to this biomarker.

The increased levels of MR-proANP, MR-proADM and CT-proAVP reflect activation of their mature counterparts. Difficulties in measurement of the mature hormones made it difficult to determine their roles during sepsis [5–7]. The currently investigated biomarkers are produced after proteolytic processing of the same prohormone precursors as the mature hormones; thus, they reflect production levels of mature hormones in stoichiometric concentrations [7, 15]. In theory, the biomarker assays may measure levels of both cut prohormone fragments and full-length prohormones. However, the meaning of this relationship remains unclear in plasma because no well-defined methods are currently available for measurement of full-length prohormone plasma levels.

The biomarker levels in this study were dose-dependently influenced by corticosteroid intervention, which is important for treatment effect monitoring. The development of new biomarkers is needed for improvement of sepsis stratification methods, which influence trial outcomes. Several sepsis intervention strategies have shown promise [16]; in this study corticosteroids were selected because they aim primarily at inhibition of the immune response, and for this intervention in particular, effects are dose-dependent and improvement of patient stratification methods is urgently needed [11, 17]. Prednisolone is among the most frequently applied corticosteroids [18]; the oral route is convenient and potentially safer than intravenous administration [19].

The doses of prednisolone used in our model were chosen according to common dose regimens for prednisolone [18]. These doses were relatively low (equivalent to 12, 40 and 120 mg hydrocortisone) in comparison to doses used for sepsis intervention (hydrocortisone 200–300 mg per day), yet sufficient to reduce the release of the investigated biomarkers during endotoxemia. Of note, it has been demonstrated that the effects of orally administered doses of prednisolone do not differ from those of comparable doses of intravenously administered hydrocortisone [20].

The human endotoxemia model differs from sepsis in many aspects: it uses a single-dose drug intervention and bolus intravenous challenge of LPS, it is self-limiting, and it lacks an infectious source [13]. The study subjects are healthy young people not using any co-medication. The intervention must be administered before LPS injection in order to reach clinically relevant drug concentrations during endotoxemia because LPS is extremely quickly cleared from the circulation. Due to these differences, data cannot be directly extrapolated to the sepsis field and must always be confirmed in patient studies. Nonetheless, the model is the best available in healthy humans and has proven useful to obtain a proof of principle of the actions of drugs and/or to study mechanisms that contribute to the activation of inflammatory pathways [13]. With these limitations in mind, we demonstrate here that the levels of the novel biomarkers MR-proADM, MR-proANP, CT-proAVP and PCT are all increased after LPS injection, and that the levels of MR-proADM, MR-proANP and CT-proAVP can be inhibited by prednisolone. These results add to their potential as biomarkers in sepsis.

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