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Altered adrenal and gonadal steroids biosynthesis in patients with burn injury

Maria Bergquist [a], Fredrik Huss [b,d], Filip Fredén [c,d], Göran Hedenstierna [a], Johanna Hästbacka [e], Alan L. Rockwood [f,g], Mark M. Kushnir [f,g], Jonas Bergquist [g,h,*]

[a] Department of Medical Sciences, The Hedenstierna Laboratory, Uppsala University, Uppsala, Sweden
[b] Department of Surgical Sciences, Plastic Surgery, Uppsala University, Uppsala, Sweden
[c] Department of Surgical Sciences, Anaesthesiology and Intensive Care, Uppsala University, Uppsala, Sweden
[d] Uppsala Burn Center, Uppsala University Hospital, Uppsala, Sweden
[e] Intensive Care Medicine Department of Perioperative, Intensive Care and Pain Medicine, University of Helsinki and Helsinki University Hospital, Finland
[f] ARUP Institute for Clinical & Experimental Pathology, 500 Chipeta Way, Salt Lake City, UT 84108-1221, USA
[g] Department of Pathology, University of Utah School of Medicine, Salt Lake City, USA
[h] Department of Chemistry – BMC, Analytical Chemistry, Uppsala University, P.O. Box 599, SE-751 24 Uppsala, Sweden

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ABSTRACT

Introduction: Burn injury inevitably leads to changes in the endogenous production of cytokines, as well as adrenal and gonadal steroids. Previous studies have reported gender-related differences in outcome following burn injury, which suggests that gonadal steroids may play a role. The aim of this study was to assess alterations in concentration of endogenous steroids in patients with burn injury.
Methods: For this single-center, prospective descriptive study, high-sensitivity liquid chromatography tandem mass spectrometry (LC-MS/MS)-based steroid quantification was used to determine longitudinal profiles of the concentrations of endogenous steroids in plasma from sixteen adult male patients with burn injury (14.5–72% of total body surface area). Steroids were extracted from plasma samples and analyzed using multiple reaction monitoring acquisition, with electrospray ionization on a triple quadruple mass spectrometer. Total protein concentration was measured in the samples using spectrophotometry.
Results: Steroid and total protein concentration distributions were compared to reference intervals characteristic of healthy adult men. Concentrations of the following steroids in plasma of burn injured patients were found to correlate positively to the area of the burn injury: cortisol ($r = 0.84$), corticosterone ($r = 0.73$), 11-deoxycortisol ($r = 0.72$), androstenedione ($r = 0.72$), 17OH-progesterone ($r = 0.68$), 17OH-pregnenolone ($r = 0.64$) and pregnenolone ($r = 0.77$). Concentrations of testosterone decreased during the acute phase and were up to ten-times lower than reference values for healthy adult men, while concentrations of estrone were elevated. By day 21 after injury, testosterone concentrations were increased in younger, but not older, patients. The highest concentrations of estrone were observed on day 3 after the injury and then declined by day 21 to concentrations comparable to those observed on the day of the injury.
Conclusion: Burn injury alters endogenous steroid biosynthesis, with decreased testosterone concentrations and elevated estrone concentrations, during the first 21 days after the injury. Concentrations of glucocorticoids, progestagens and androgen precursors correlated positively with the area of burn injury. The finding of increased estrone following burn injury needs to be confirmed in a larger hypothesis-driven study.

1. Introduction

Severe burn injury is associated with high mortality and multiple organ failure [1]. The stress factors after burn injury are many and continuous. Large open wounds, dressing changes, mechanical

* Corresponding author at: Department of Chemistry – BMC, Analytical Chemistry, Uppsala University, P.O. Box 599, SE-751 24 Uppsala, Sweden.
E-mail address: jonas.bergquist@kemi.uu.se (J. Bergquist).

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ventilation, surgery and infection may all cause severe inflammation. In addition, a hypermetabolic state may follow with increased metabolic rate, peripheral insulin resistance, extensive protein wasting, lean body mass loss, and bone and muscle catabolism, often complicated with functional and structural alterations of essential organs [2–4]. The altered homeostasis after burn injury inevitably leads to changes in the circulating levels of cytokines, glucagon, catecholamines, and adrenal and gonadal steroids [5,6]. Marked perturbations in HPA axis and elevated cortisol concentrations have also been observed in major burn injury [7–11]. Moreover, gender-specific differences in mortality following burn injury have been previously reported [12,13]. Among burn trauma patients, premenopausal women have a survival advantage, as compared to men and postmenopausal women [14,15]. In addition, gender and age differences have been documented in concentrations of circulating cytokine and multiple organ dysfunction syndrome (MODS), following polytrauma [16]. Physiologic concentrations of estrogens under normal conditions were suggested to be immune-stimulatory, while testosterone is thought to suppress immunity [17,18]. Hypotestosteronemia, defined as total serum testosterone < 2.5 ng/mL [19], was commonly observed in critically ill men [20–22].

Previous studies have demonstrated differences in concentrations of gonadal steroids in burn patients, but have focused only on estradiol and testosterone, and have not examined the upstream intermediates and precursors of the steroid biosynthesis pathway, (e.g., estrone, androstenedione, DHEA, pregnenolone, 17OH-pregnenolone and 17OH-progesterone). Commercially available immunoassays were shown to be unreliable for quantification of testosterone when endogenous concentrations are expected to be low [23].

This study is a single-center, prospective descriptive study of male adult patients with burn injury with the aim of assessing changes in the biosynthesis of adrenal and gonadal steroids during the acute (0–3 days) and the sub-acute (7–21 days) phases following burn injury. To our knowledge, this is the first report to assess concentration variability for a panel of endogenous steroids in patients with burn injury, using high sensitivity LC-MS/MS methods.

2. Materials and methods

2.1. Patients

The study was approved by the Ethical Committee for Human Research in Uppsala, Sweden

(Dnr 2011/484). Patients were recruited between March 2012 and March 2013 from a larger study cohort in the Burn Center (BC) of Uppsala University Hospital, Sweden. Informed consent was obtained via next of kin and directly from survivors as soon as possible. Adult patients (>18 years) were eligible regardless of burn injury type. Exclusion criteria were malignancy, immune deficiency (HIV, cytostatic drugs, corticosteroids, tetracyclines or certain bisphosphonates), known or suspected blood-transmitted infections and participation in another clinical study within the last 4 weeks. Patients were partitioned into two subgroups based on burn size: severely injured (>20% burned of the total body surface area (TBSA)) and moderately injured (<20% TBSA burned) [24]. To limit confounding factors in the study, only samples from adult men were included. Sepsis was considered present based on criteria from the American Burn Association [25]: laboratory signs of infection (e.g., reduced platelets (not caused by bleeding), increased or reduced leucocytes, increased CRP and procalcitonin (PCT)), clinical signs of infection (e.g., body temperature >39 or <36.5 °C, signs of pneumonia, obvious wound infections), and positive bacterial cultures from blood, wounds or airways. Signs of newly developed circulatory instability were also included in the sepsis assessment, such as reduced blood pressure, increasing lactate levels and need for intravenous fluid and vasoactive support.

Daily monitoring of the degree of organ dysfunction (serum albumin levels, bilirubin and creatinine levels, platelet count, Glasgow Coma Scale score, maximal vasoactive/inotrope dose, and lowest PaO_2/FiO_2) were registered and Sequential Organ Failure Assessment (SOFA-score) was determined on every sampling day. Other laboratory tests (e.g., CRP, procalcitonin, leukocyte count, and microbiological cultures) were performed as clinically needed. Patients' weight and daily cumulative fluid balance were determined on each day of sampling. Outcome (28 day and 3 month mortality) was registered for each patient.

2.2. Sample collection

Blood samples for analysis of steroids were collected from arterial line/venipuncture upon admission and in intervals thereafter at 1, 3, 7, 14 and 21 days after admission. Blood samples were centrifuged at 2000*g* for 10 min, plasma was separated and stored at −70 °C until analysis.

2.3. Steroids analysis

Five classes of steroids were measured in plasma samples using LC-MS/MS: glucocorticoids (cortisol, cortisone, corticosterone, 11-deoxycortisol); androgens (dehydroepiandrosterone (DHEA), androstenedione, testosterone); pregnanes (pregnenolone, 17-OH pregnenolone); estrogens (estrone and estradiol); and progestins (17-OH progesterone, progesterone).

Testosterone (Te), estrone (E1), estradiol (E2), pregnenolone (Pregn), cortisol, cortisone, 17-hydroxypregnenolone (17OHPregn), 17-hydroxyprogesterone (17OHP), formic acid, hydroxylamine, trifluoroacetic acid, sodium carbonate and dansyl chloride were purchased from Sigma Chemical Company (St Louis, MO, USA).

Androstenedione (A4), dehydroepiandrosterone (DHEA) and progesterone (PROG) were purchased from Steraloids Inc. (Newport, RI, USA). Deuterium labeled analogues of the steroids d_3-testosterone, d_3-pregnenolone, d_2-11-deoxycortisol, d_9-17-OH progesterone, d_3-17OH pregnenolone, d_4-cortisol, d_3-cortisone, (purchased from Cambridge Isotope Laboratories, Andover, MA, USA), and d_4-estrone and d_3-estradiol, (purchased from CDN Isotopes, Toronto, ON, Canada) were used as internal standards. Calibration standards were prepared using standards purchased from Cerilliant (Round Rock, TX, USA). All other chemicals were of the highest purity commercially available.

Samples were analyzed as previously described [26–29]. In short, steroids were extracted from 100 μL of plasma aliquots; cortisol and corticosterone were analyzed as described in [30]; DHEA, A4, Te, Pregn, 17OHPregn, 17OHP and progesterone (P4) were derivatized with hydroxylamine to form oxime derivatives; estrone and estradiol were derivatized with dansyl chloride to form dansyl derivatives [27]. Limits of quantification (LOQ) are presented in Table 1 [28]. The intra-assay and inter-assay CVs were <8% and <11%, respectively [26–28]. All steroids were analyzed in positive ion mode using an electrospray ion source on a triple quadruple mass spectrometer (AB Sciex 5500; Foster City, CA, USA). The HPLC system consisted of series 1260 and 1290 HPLC pumps (Agilent Technologies, Santa Clara, CA, USA), and an HTC PAL autosampler (LEAP Technologies, NC, USA) equipped with a fast wash station. Two mass transitions were monitored for each steroid and its internal standard (IS). Quantitative data analysis was performed using Analyst® 1.5.2 software. Calibration curves were generated with every set of samples using six calibrators. Three quality control samples were included with every set of sam-