Assessment of tissue cortisol activity

Jeremy Cohen and Bala Venkatesh

ABSTRACT

The concept of relative adrenal insufficiency in patients with severe sepsis continues to be controversial. This arises in part from the lack of an accepted "gold standard" for the diagnosis of adrenal insufficiency in the critically ill. Historically, assessment of adrenal function in this population has relied on measurement of plasma total cortisol level, in a blood sample taken either at random or as part of a corticotropin stimulation test. However, an alternative is to focus on the site of glucocorticoid activity within the tissues as a potentially more useful index of functional adrenal status.

We review the mechanisms known to affect tissue glucocorticoid activity and examine how they may be modified by critical illness. These include both free and interstitial cortisol concentrations, intracellular cortisol generation, and glucocorticoid-receptor activity and density. Changes in these factors are not reflected in plasma total cortisol concentrations, and more sophisticated techniques, including genetic transcriptional surveys, may be required to reveal the role of glucocorticoid insufficiency in critical illness.

Crit Care Resusc 2009; 11: 287–289

For editorial comment, see page 235. See also page 301.

Standard methods of assessing adrenal activity based on measuring plasma cortisol concentration have been shown to suffer numerous problems, including lack of reproducibility,[1] large interassay variation,[2] lack of agreement on diagnostic criteria, excessive variation, and poor correlation with outcome.[3,4] As a result, it has proven difficult to reliably identify patients who have suppressed adrenal activity and therefore may benefit from administration of exogenous corticosteroids.

An alternative approach to this problem is to focus on the site of action of cortisol in the tissues as likely to reflect adrenal function more accurately. Recent research has shown a number of mechanisms by which glucocorticoid activity might be modulated at a tissue level. An understanding of these mechanisms, and how they change in critical illness, might allow us clearer insight into how to measure adrenal function in this population.

Glucocorticoids, of which cortisol is the primary example in humans, circulate in both bound and free forms. At normal levels of circulating cortisol, over 95% is bound to an α_2-globulin, cortisol-binding globulin, with a small fraction bound to albumin. The remainder circulates as a free fraction, and it is this portion that is biologically active.

Free plasma cortisol passes from the plasma to the interstitium, and then through the cell membrane. Within the cell, cortisol is subject to the action of the 11β-hydroxysteroid dehydrogenase (11β-HSD) enzyme system. This enzyme system exists as two isomers, 11β-HSD1 and 11β-HSD2, which catalyse the interconversion of active cortisol and inactive cortisone. Cortisol subsequently binds to the glucocorticoid receptor, a cytoplasmic protein found in nearly all nucleated cells. Once cortisol binds to the receptor, its ligand-binding domain undergoes a conformational change, shedding heat shock factors and migrating to the nucleus, where the receptor binds to glucocorticoid response elements and influences gene transcription. It is readily apparent that modification of any of these steps could alter the expression of glucocorticoid activity, independently of circulating total plasma cortisol concentration. These modifications include changes in plasma free cortisol levels, factors influencing interstitial cortisol concentrations, 11β-HSD activity, glucocorticoid receptor concentrations, binding affinity, and translocation. There is evidence to suggest that severe sepsis may influence most, if not all, of these factors.

Plasma free cortisol

Most studies of adrenal function in patients with sepsis have measured concentrations of total plasma cortisol, which includes both bound and free forms. Plasma free cortisol levels can vary because of changes in concentration or binding affinity of cortisol-binding globulin, without a measured change in total plasma cortisol. Currently, neither plasma free cortisol nor cortisol-binding globulin measurements are generally available to the practising clinician.

The limited available data suggest that plasma free cortisol is a more accurate marker of adrenal function in the critically ill than total plasma cortisol. Hamrahian et al[5] found, in 66 critically ill patients, that there was a greater relative increase in free compared with total plasma cortisol concentrations. Patients with hypoproteinaemia had lower total plasma cortisol concentrations than patients without hypoproteinaemia, whereas plasma free cortisol concentrations did not differ significantly between the two groups. Similarly, Ho et al investigated 74 patients with sepsis or septic shock and showed that plasma free cortisol concentrations better reflected illness severity than total plasma cortisol concentrations.[6]

We have collected data from our institution that indicate plasma free cortisol shows a relatively greater response to a low-dose corticotropin stimulation test than total plasma cortisol, suggesting free cortisol is a more sensitive measure for diagnosing adrenal insufficiency. However, no large-scale out-

come studies have as yet compared plasma free cortisol with plasma total cortisol as a marker of adrenal insufficiency in patients with septic shock, and its superiority has not been definitively established.

Interstitial cortisol concentrations

Interstitial cortisol concentrations reflect the glucocorticoid pool available to pass through the cell membrane and bind to the glucocorticoid receptor. Although these concentrations are closely related to the plasma free cortisol concentration, other factors are likely to have an influence. Neutrophil elastase, an enzyme released from polymorphonuclear leukocytes, cleaves cortisol from cortisol-binding globulin, and thus may increase local interstitial cortisol levels at sites of inflammation.[7] Additional mechanisms include changes in interstitial fluid volume, increased capillary "leakage" and reduced peripheral tissue perfusion, all of which are likely to occur in patients with sepsis.

We undertook a pilot study to measure interstitial cortisol levels using a microdialysis technique in critically injured patients with severe burns (unpublished data). These data revealed significantly increased interstitial cortisol concentrations that were poorly correlated with plasma free cortisol values. To our knowledge, this is the first study of its type in the intensive care population and appears to support the hypothesis that plasma cortisol concentrations are not the sole determinant of glucocorticoid availability to the tissues.

11β-hydroxysteroid dehydrogenase

The recognition of the 11β-HSD enzyme system has profoundly affected our understanding of glucocorticoid physiology. Of the two isoforms of this enzyme, 11β-HSD1 appears to be primarily a reductase, converting inactive cortisone to cortisol, thus generating active glucocorticoid at a pre-receptor level. Conversely, 11β-HSD2 functions physiologically only in the dehydrogenase mode, catalysing the conversion of cortisol to cortisone. Its major site of action is the kidney, where it functions to inactivate cortisol before the latter binds and activates the mineralocorticoid receptor.

Exponentially increasing research in the past decade on the 11β-HSD system has confirmed its central place in glucocorticoid metabolism. Abnormalities of the system have been implicated in the pathogenesis of obesity, hypertension, and the metabolic syndrome.[8] Genetically modified mice that produce excess 11β-HSD1 develop obesity, hypertension and dyslipidaemia; conversely, knockout 11β-HSD1 mice are resistant to these conditions. However, despite the interest in this field, very few studies have examined the role of the 11β-HSD system in critical illness.

Some of the difficulty in this field arises from the fact that the enzyme system is intracellular, and its activity is tissue-dependent. However, an index of overall 11β-HSD activity can be obtained by examining the ratio of total plasma cortisol to total plasma cortisone. This ratio gives information on the "set point" of the 11β-HSD equilibrium, indicating if it is in favour of cortisol generation or inactivation.

Previous research showed an increase in the cortisol to cortisone ratio in postoperative cardiac patients, as well as a correlation between the cortisol to cortisone ratio and C-reactive protein level in a non-selected general hospital population.[9,10] We published the first data examining cortisol to cortisone ratios in patients with critical illness and showed that these ratios are significantly elevated in those with sepsis and trauma.[11] This suggests an increase in intracellular cortisol generation in this cohort of patients, independent of circulating plasma cortisol concentration. Further support for this hypothesis comes from recent data from our laboratory examining tissue expression of 11β-HSD1 in an animal model of sepsis. Samples of liver and adipose tissue from rats that had undergone laparotomy and caecal perforation (sepsis model) were compared with tissue from control rats. Quantitative analysis of 11β-HSD1 expression showed highly significant increases in rats with sepsis compared with control rats.

Alterations in the glucocorticoid receptor

The glucocorticoid receptor is part of the nuclear hormone receptor superfamily of ligand-activated transcription factors. Only one gene is known to encode for the receptor, located on chromosome 5. Two isomers of the receptor have been described, GRα and GRβ. The latter is generated by alternative splicing at the ninth exon, and does not bind ligand. Because of this functional inactivity, it was initially thought to be a minor gene product, but it is now believed to act as a primary inhibitor of GRα. Increased expression of GRβ in patients with rheumatoid arthritis is associated with glucocorticoid resistance. However, the glucocorticoid receptor gene is mostly expressed as the GRα isomer, which is the functionally active form. It appears that the receptor undergoes post-translational modification, which significantly affects its function.

Before binding to glucocorticoid, the cytoplasmic glucocorticoid receptor forms a multiprotein complex with several proteins, including heat-shock proteins, co-chaperones, and various protein kinases. Binding of glucocorticoid to the ligand domain of the receptor results in dissociation from the multiprotein complex and translocation of the receptor-ligand complex into the cell nucleus. Translocation occurs within 30 minutes of exposure of the cell to glucocorticoid and results in binding to specific DNA binding sites, termed glucocorticoid response elements (GREs). GREs can have positive or negative effects on transcription, affecting an estimated 2000 genes.

Sepsis and critical illness may significantly affect glucocorticoid receptor function. A decrease in GRα-binding capacity has been noted in a sheep model of acute lung injury.[12] Pariante and colleagues showed that interleukin-1α reduced glucocorticoid receptor translocation and glucocorticoid receptor-medi-

ated gene transcription in a mouse fibroblast cell line,[13] while tumour necrosis factor α has also been shown to impair glucocorticoid receptor-mediated transcription.[14] Meduri et al showed a reduction in GRα nuclear staining in immunohistochemical analysis of lung tissue from patients with acute respiratory distress syndrome (ARDS) that was not resolving.[15] In a study examining peripheral blood monocytes from patients with sepsis, Molijn et al did not observe a decrease in glucocorticoid-receptor numbers, but did show decreased binding affinity.[16]

Glucocorticoid-regulated gene expression

It can be seen from the preceding review that the available evidence does not allow a clear statement of the effects of critical illness on tissue glucocorticoid activity. Observed changes in plasma free cortisol and interstitial cortisol, and evidence of upregulation of 11β-HSD1, indicate increased glucocorticoid activity; conversely, the effect on glucocorticoid receptor numbers and action appears to be inhibitory. A solution to this apparent impasse may be to examine the "final pathway" of glucocorticoid activity — targeted gene expression.

Recent studies using microarray analysis and real-time quantitative polymerase chain reaction tests have allowed the primary target genes for glucocorticoid receptor to be identified in human cell lines.[17] This potentially allows the conduct of genome-wide transcriptional surveys to identify how strongly cortisol signals register on the entire transcriptome. Studies using this technique have investigated the possibility of tissue glucocorticoid resistance in patients suffering chronic psychological stress.[18] To date, there have been no similar studies in patients with critical illness.

Conclusions

There is extensive evidence to suggest that, in critical illness, a number of mechanisms can influence the expression of cortisol activity at a tissue level, and this may be a reason for the observed inadequacy of total plasma cortisol as a reliable marker of adrenal function is this setting.

However, these mechanisms may have competing effects in either increasing or decreasing glucocorticoid expression, and their clinical relevance and magnitude has yet to be determined. Examining the final common pathway of gene expression may be a potentially useful technique in resolving this complex question.

Author details

Jeremy Cohen, Staff Specialist in Intensive Care[1]
Bala Venkatesh, Professor of Intensive Care[2]
1 Burns Trauma and Critical Care Research Centre, University of Queensland, Brisbane, QLD.
2 Princess Alexandra and Wesley Hospitals, and University of Queensland, Brisbane, QLD.
Correspondence: jeremy_cohen@health.qld.gov.au

References

1 Loisa, P, A Uusaro, E Ruokonen. A single adrenocorticotropic hormone stimulation test does not reveal adrenal insufficiency in septic shock. *Anesth Analg* 2005; 101: 1792-8.
2 Cohen J, Ward G, Prins J, et al. Variability of cortisol assays can confound the diagnosis of adrenal insufficiency in the critically ill population. *Intensive Care Med* 2006; 32: 1901-5.
3 Venkatesh B, Mortimer RH, Couchman B, Hall J. Evaluation of the random plasma cortisol and the low dose corticotropin test as indicators of adrenal secretory capacity in critically ill patients. A prospective study. *Anaesth Intensive Care* 2005; 33: 201-9.
4 Cooper MS, Stewart PM. Corticosteroid insufficiency in acutely ill patients. *N Engl J Med* 2003; 348: 727-34.
5 Hamrahian AH, Oseni TS, Arafah BM. Measurements of serum free cortisol in critically ill patients. *N Engl J Med* 2004; 350: 1629-38.
6 Ho JT, Al-Musalhi H, Chapman MJ, et al. Septic shock and sepsis: a com-parison of total and free plasma cortisol levels. *J Clin Endocrinol Metab* 2006; 91: 105-14.
7 Pemberton PA, Stein PE, Pepys MB, et al. Hormone binding globulins undergo serpin conformational change in inflammation. *Nature* 1988; 336: 257-8.
8 Tomlinson JW, Tomlinson JW, Walker EA, et al. 11beta-hydroxysteroid dehydrogenase type 1: a tissue-specific regulator of glucocorticoid response. *Endocr Rev* 2004; 25: 831-66.
9 Vogeser M, Groetzner J, Kupper C, et al. The serum cortisol:cortisone ratio in the postoperative acute-phase response. *Horm Res* 2003; 59: 293-6.
10 Vogeser M, Zachoval R, Felbinger TW, Jacob K. Increased ratio of serum cortisol to cortisone in acute-phase response. *Horm Res* 2002; 58: 172-5.
11 Venkatesh B, Cohen J, Hickman I, et al. Evidence of altered cortisol metabolism in critically ill patients: a prospective study. *Intensive Care Med* 2007; 33: 1746-53.
12 Liu LY, Sun B, Tian Y, et al. Changes of pulmonary glucocorticoid receptor and phospholipase A2 in sheep with acute lung injury after high dose endotoxin infusion. *Am Rev Respir Dis* 1993; 148 (4 Pt 1): 878-81.
13 Pariante CM, Pearce BD, Pisell TL, et al. The proinflammatory cytokine, interleukin-1alpha, reduces glucocorticoid receptor translocation and function. *Endocrinology* 1999; 140: 4359-66.
14 Kino T, Chrousos GP. Tumor necrosis factor alpha receptor- and Fas-associated FLASH inhibit transcriptional activity of the glucocorticoid receptor by binding to and interfering with its interaction with p160 type nuclear receptor coactivators. *J Biol Chem* 2003; 278: 3023-9.
15 Meduri GU, Muthiah MP, Carratu P, et al. Nuclear factor-kappaB- and glucocorticoid receptor alpha- mediated mechanisms in the regulation of systemic and pulmonary inflammation during sepsis and acute respiratory distress syndrome. Evidence for inflammation-induced target tissue resistance to glucocorticoids. *Neuroimmunomodulation* 2005; 12: 321-38.
16 Molijn GJ, Koper JW, van Uffelen CJ, et al. Temperature-induced down-regulation of the glucocorticoid receptor in peripheral blood mononuclear leucocyte in patients with sepsis or septic shock. *Clin Endocrinol (Oxf)* 1995; 43: 197-203.
17 Wang JC, Derynck MK, Nonaka DF, et al. Chromatin immunoprecipitation (ChIP) scanning identifies primary glucocorticoid receptor target genes. *Proc Natl Acad Sci U S A* 2004; 101: 15603-8.
18 Miller GE, Chen E, Sze J, et al. A functional genomic fingerprint of chronic stress in humans: blunted glucocorticoid and increased NF-kappaB signaling. *Biol Psychiatry* 2008; 64: 266-72. ❑