Steroids and nitric oxide in sepsis

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1. ABSTRACT

Nitric oxide (NO) is produced by several cell types and has effects both detrimental and beneficial to the host. Sepsis and septic shock are conditions in which NO plays a central role in physiopathology. Stressful circumstances such as pathogens, toxins, and trauma elicit a wide variety of physiological changes. Steroid hormones and notably glucocorticoids are one of the main players in this orchestrated response. Although steroids have been used for sepsis some decades ago, their use in this condition was practically banned for several years following studies showing that high glucocorticoid doses were harmful to the host. Recently, the subject has been raised again since some studies demonstrated that adrenal insufficiency may happen in sepsis and that low dose/long-term regimen with cortisol may be beneficial to sepsis and septic shock. However, there are great gaps in our knowledge regarding the role played by steroids in sepsis, as well as the contribution of NO. In the present review, we will attempt to highlight the relationship among NO, sepsis and steroids, mainly glucocorticoids. A second purpose is to raise some unanswered questions that may provide better therapeutic alternatives to treat sepsis and septic shock.

2. INTRODUCTION

Nitric oxide (NO) is produced by several cell types and has effects both detrimental and beneficial to the host. Sepsis and septic shock are conditions in which NO plays a central role in physiopathology. Since a comprehensive description of NO biology is out of the scope of this review, the reader is referred to some authoritative reviews on this subject (1-7). In humans and other vertebrates, stressful circumstances such as pathogens, toxins, and trauma elicit a wide variety of physiological changes that constitute the so-called "stress response". One of the main players in this orchestrated response are steroid hormones, mainly glucocorticoids. The influence of these hormones are wide in the organism and critical for survival in a normal (as well as in a stressful) environment. The reader interested in in-depth information on steroids is referred to comprehensive reviews (8-12).

The main purpose of this review is to highlight the relationship among NO, sepsis and steroids, mainly glucocorticoids. Space limitations do not allow for detailed discussion of many relevant articles for which we apologize to all those colleagues that have not been cited.

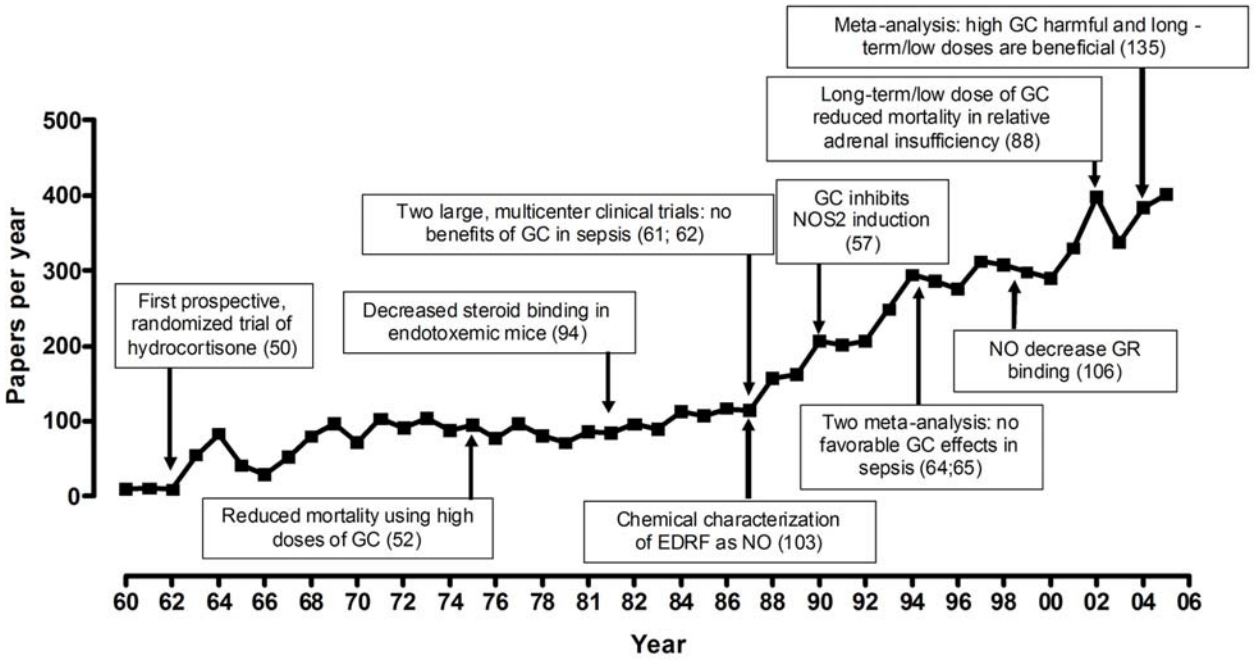

Figure 1. Time-line of seminal papers relevant to the present review. Also depicted is the number of published papers on the field per year from 1960 to 2006. The search was performed in PubMed and the strategy was [(corticoids OR steroids OR glucocorticoid) AND (sepsis OR "septic shock" OR lipopolysaccharide OR endotoxin)]. Numbers in parenthesis refer to the reference list.

In order to help the reader to identify the seminal papers supporting the present review, a time-line is presented in Figure 1. In the same Figure, it is presented the number of papers published since 1960 in this subject. As can be easily seen, the subject of the present review is getting more and more attention from the clinical and academic communities.

3. PHYSIOLOGY OF STEROIDS

3.1. Introduction

The connection between stress and adrenocortical hormones was revealed in the 1930s by findings that stress stimulates adrenocortical secretion (12; 13). Two major types of adrenocortical hormones are secreted by the adrenal glands, the mineralocorticoids (MC) and the glucocorticoids (GC). These hormones are synthesized from cholesterol, and they all have similar chemical structure, but slight differences in their molecular structures give them several different functions.

Named for their role in maintaining glucose homeostasis, GC influence the activity and direction of a myriad of cell-, tissue- and organ-specific functions including intermediary metabolism (glucose homeostasis, protein, lipid and carbohydrate metabolisms), maintenance of vascular tone, immune and inflammatory regulation, effects on the central nervous system (arousal, cognition, mood and sleep), development and programmed cell death (for review see 10; 14).

The increase of production/release of cortisol acutely shifts carbohydrate, fat, and protein metabolisms so that the energy is instantly and selectively available to vital organs, such as the brain. In response to hypercortisolism, amino acids from peripheral tissues (such as skeletal muscle) are mobilized to liver, leading to increase of gluconeogenesis and consequently glucose offer (10). The increase of cortisol production in response to an acute disease, trauma, surgery, or sepsis, can be interpreted as an attempt of the organism to mute its own inflammatory cascade, thus protecting itself against over responses.

Concomitantly to hypercortisolim, circulating aldosterone (which is the main mineralocorticoid) increases markedly, most likely under the control of an activated renin-angiotensin system (15). The increase of aldosterone production affects electrolytes of extracellular fluid (in particular sodium and potassium). Without the mineralocorticoids, potassium ion concentration of the extracellular fluids rises markedly, sodium and chloride concentrations decrease, and total extracellular fluid volume and blood volume also become greatly reduced.

Thus, in this part of the review we will discuss briefly the synthesis and release of steroids, their receptors and some of their effects, relevant for sepsis.

3.2. Activation of the hypothalamic-pituitary-adrenal (HPA) axis

Circulating GC concentrations are largely under the control of HPA axis. The main hypothalamic regulator of the HPA axis, corticotropin-releasing hormone (CRH) is produced by cells of the paraventricular nucleus of the hypothalamus and secreted into the hypophyseal blood supply around the pituitary gland. This stimulates the

release of adrenocorticotropic hormone (ACTH) from the anterior pituitary into the blood. In the adrenal cells, ACTH interacts with specific receptors on plasma membrane leading to production and release of cortisol from the adrenal cortex. Arginine-vasopressin (AVP) is also released from the hypothalamus, acting synergistically with CRH to induce ACTH secretagogue activity (16). This axis is self-regulated. Glucocorticoids exert a classic feedback inhibitory effect on the production of CRH/AVP and ACTH. The inhibition of CRH/AVP and the ACTH secretion serves to limit the duration of the total tissue exposure to GC, thus minimizing the catabolic, lipogenic, anti-reproductive, and immunosuppressive effects of these hormones (for review see 17).

Many bacterial and viral infections result in activation of HPA axis and increased glucocorticoid release. In animals, injection of endotoxin (bacterial lipopolysaccharide; LPS) activates HPA axis and increases ACTH and glucocorticoid levels (18). During immune responses, certain cytokines can signal to the central nervous system, which through a complex CRH-dependent pathway triggers activation of HPA axis. Most of the HPA-axis stimulating activity in plasma comes from three cytokines, TNF-α, IL-1 and IL-6, which are produced at inflammatory sites and elsewhere in response to inflammation. All three cytokines independently activate the HPA axis; in combination, their effects are synergistic (19; 20). They also directly stimulate CRH secretion in rat hypothalamus explants, and this effect can also be blocked by glucocorticoids. The three inflammatory cytokines also mediate the stimulatory effect of LPS on the HPA axis. The HPA axis provides an important physiological feedback loop in inflammation through the anti-inflammatory effects of glucocorticoids.

3.3. Synthesis and release

Synthesis and secretion of GC is controlled by neural and humoral signals that change throughout the day and respond to stress and negative feedback. The synthesis of GC in the adrenal cortex is closely tied to plasma levels of ACTH, which exhibit episodic peaks and circadian rhythm similar to plasma levels of GCs. Two or three synchronous pulses of CHR and AVP are released from the hypothalamus into the hypophyseal portal system every hour. In early morning, the amplitude of these pulses is maximal, increasing the amplitude and frequency of ACTH and cortisol secretory episodes. The amplitude of CRH and AVP pulses also increases during the acute stress, resulting in increases in the amplitude and apparent frequency of ACTH and cortisol pulses (reviewed in 10).

ACTH stimulates steroidogenesis by binding to membrane receptors on adrenal cells, which activates adenylate cyclase and also causes hypertrophy and hyperplasia of the adrenal cortex. Most of the secreted GC (approximately 90%) are bound to corticosteroid-binding globulins or transcortin in the blood. On the other hand, aldosterone combines only loosely with the plasma proteins so that about 50 percent is in free form. In both combined and free forms, hormones are transported throughout the extracellular fluid compartment. Due to the high lipophylic nature of steroid molecules, these hormones can reach virtually all tissues in the organism including brain and readily diffuse across the cell.

3.4. Receptors and mechanism of action

Glucocorticoids readily penetrate the cell membrane and interact with ubiquitous cytoplasmatic and nuclear glucocorticoid receptors (GR), through which they exert their effects (8; 21). GR is a member of the large super-family of nuclear receptors that function as ligand-dependent transcription factors. This family also includes estrogen receptors, androgen receptors, progesterone and mineralocorticoids receptors, as well as receptors for vitamin D, thyroid hormone retinoic acid, the peroxisome-proliferator-activated receptors, and a large group of orphan receptors whose ligands have not yet been identified (22; 23).

The human GR gene, located in the chromosome 5 (23) was originally cloned in 1985 and consist of nine exons (24). This structural organization produces two isoforms of GR, GRα and GRβ, by the alternative splicing of exon 9. The hGRβ does not itself bind glucocorticoids, but rather exerts dominant negative effects on GRα through several mechanisms, such as heterodimerization and competition with GRα for GRE (glucocorticoid response elements) or transcriptional nuclear coactivators (or both) (25). The over expression of GRβ isoform in mouse hybridoma cells generate a GC-resistant phenotype (26). The increased expression of hGRβ has been identified as a contributing factor to tissue-specific glucocorticoid resistance in several pathological conditions (27; 28). For example, increased hGRβ levels have been reported in T cells in the airway, peripheral blood mononuclear cells, and in tuberculin-induced inflammatory lesions in glucocorticoid-insensitive asthmatics (8). Therefore, an imbalance in the relative levels of hGRα and hGRβ may underlie the pathogenesis of several clinical conditions associated with glucocorticoid resistance, such as rheumatoid arthritis, systemic lupus erythematosus, or ulcerative colitis (29).

Similar to other steroid receptors, GR is a modular protein organized into three major functional domains that include a variable-length N-terminal domain (NTD), a central two zinc finger DNA-binding domain (DBD), and the C-terminal ligand-binding domain (LBD) (30). In addition, GR has sub-domains; two transcription activation regions AF-1 (function dependent of ligand binding) and AF-2 (ligand binding dependent), present in the NTD and LBD, respectively. The LBD also has a nuclear localization signals and sites for interaction with other transcription factors, cofactors, or chaperones (31).

The classical mode of action of glucocorticoids and others steroid hormones occur through the direct regulation of gene transcription. In the absence of ligand, GR is located in the cytoplasm in a large multi-protein complex that includes two molecules of heat shock protein 90, heat shock protein 70, immunophilins, FKBPs (FK506 binding protein), Cyp-40 (cyclophilin 40), p23, and possibly a few others (32; 33). These proteins assist in proper GR folding, and maintain GR in a transcriptionally

inactive state that is ready to bind to hormone. Upon hormone binding, the receptor undergoes conformational changes leading to its dissociation from the cytoplasmatic chaperones and exposure of its nuclear localization signals. In this new conformation, ligand-bound GR translocates to the nucleus (34) and once inside, GR homodimers readily recognize and interact with GREs located in the regulatory regions of target genes. GR is able to up-regulate gene expression through direct DNA binding, for example, it binds to the glucogenic enzyme tyrosine amino transferase gene, whose promoter contains a consensus GRE sequence. GR can also repress gene activation trough binding to negative GREs, such as the pro-opiomelanocortin gene (35). GR can also exert its repressive effects by interfering with the action of other signaling pathways, such as nuclear factor kappa B (NF-κB) and activator protein-1 (AP-1). It is through this mechanism that GCs exert many of their anti-inflammatory mechanism (36). Since the first description of this mechanism in 1990 for AP-1 (37), an increased number of transcription factors, such as Sma and Mad-related protein (Smad), signal transduction and activator of transcription (STAT) and cAMP response element-binding protein (CREB) have been demonstrated to physically interact with GR (8). In response to pro-inflammatory cytokines, bacterial and viral infection agents and pro-apoptotic stimuli the transcription factors NF-κB and AP-1 are activated, leading to up-regulation of pro-inflammatory genes. The production of the pro-inflammatory machinery can be attenuated by interaction of GR with the p65 subunit leading to repression of NF-κB transcriptional activity. The same mechanism is proposed to repress the transcriptional activity of AP-1. Interestingly, the repressive effect of GR on NF-κB regulation seems to be mutual, since NF-κB has also been shown negatively regulate GRα-mediated transcription (38).

The notion that GR is essential for life, is underscored by the finding that GR knockout mice suffer from a lung defect and die shortly after birth. In contrast, GR dimerization-deficient mice ($GR^{dim/dim}$) that cannot bind to GREs and consequently cannot mediate GRE-dependent gene activation are still viable. In these mice, heterodimerization with other transcription factors like NF-kB and AP-1 remains intact, suggesting that the anti-inflammatory actions of GR that are thought to be mediated by its interference with other signaling pathways through protein-protein interactions may be most critical for survival (39).

The human mineralocorticoid receptor (MR) is closely related to GR, with 94% aminoacid identity in the DBD and 57% in the C-terminal LBD. MR is located on the chromosome 4q31.2 and in contrast to GR, MR has a tissue-specific pattern of expression with the highest levels observed in the distal nephron, distal colon and hippocampus (40). Similar to GR the unliganded MR is predominantly cytoplasmatic, being complexed with chaperones. In response to ligand, MR translocate to the nucleus and initiate the gene transcription. Mice homozygous for inactivating mutations in the MR gene (41) show classical features of aldosterone deficiency, such as salt-wasting, hyperkalemia, and dehydration. However, treatment by salt supplementation allows survival and normal growth.

3.5. General and anti-inflammatory effects

Aldosterone and other mineralocorticoids acts on epithelial cells, particularly in the kidney, but also in salivary glands and colon. Although there are others, the main mediators of aldosterone effects are the ouabain-sensitive serosal Na/K-ATPase and the luminal amiloride-sensitive epithelial sodium channel. The net result is sodium (and water) retention and potassium increased excretion. Aldosterone also have non-epithelial effects in the cardiovascular and central nervous systems, that may be important in the sepsis context. For example, it can modulate vascular tone by increasing the pressor response to catecholamines or upregulating angiotensin II receptors (reviewed in 42; 43).

The classical metabolic effects of glucocorticoids include increases in blood glucose levels by promoting insulin resistance, and hepatic gluconeogenesis and glycogenolysis, of which glycogen synthesis is by far the most important. Other effects of this class include enhancement of lipolysis and proteolysis. The increased amino acids pool is fueled for glucose production and storage as glycogen in the liver (reviewed in 44; 45).

Glucocorticoids are well known for their powerful anti-inflammatory and immunosuppressive actions. Anti-inflammatory effects of glucocorticoids include decreases in vascular permeability, leukocyte adhesion and migration, cytokine synthesis and release, synthesis and release of inflammatory mediators, expression of pro-inflammatory proteins and enzymes (such as NOS-2 and COX-2) and tissue destruction (reviewed in 44; 46).

4. STEROIDS AND SEPSIS

4.1. Introduction

The initial phase of sepsis is associated with an intense inflammatory activity. The powerful anti-inflammatory effect of glucocorticoids naturally led to the proposal of their use in sepsis.

4.2. Early studies

Indeed, glucocorticoids are one of the oldest therapies to have been considered for this syndrome in human studies, dating back to the early 1940s (47). Early reports clearly demonstrated the improvement of hemodynamic stability induced by corticoids (48). As hydrocortisone seemed to be helpful to 3 patients in septic shock, the use of massive doses of steroids in sepsis was suggested (49). The first prospective, randomized trial of hydrocortisone vs. placebo in patients with severe sepsis or septic shock was published in 1963 (50). The authors did not find any significant difference in survival between treatment and control arms. High doses of glucocorticoids increased cardiac output in a group of 9 patients with acute hypotension due to sepsis (51). A very impressive reduction in mortality (from 38% to 10%) was demonstrated in patients with septic shock treated with

steroids (52). Thus, based on these studies and others, high doses of glucocorticoids in sepsis became a standard practice in the late 1970s and early 1980s. Many investigations were stimulated by these pioneering works, and several experimental reports have showed positive effects of high doses of steroids in sepsis and septic shock. For instance, glucocorticoids have been shown to prevent several endotoxin effects, such as activation of the complement system (53), increased aggregation and adhesion of leukocytes (54; 55), prostaglandin generation (56), expression of the inducible form of nitric oxide synthase (57; 58) and transcription of TNF-alpha and other pro-inflammatory cytokines (59; 60).

In spite of these several positive reports, two large randomized clinical studies of high doses of glucocorticoids revealed their lack of efficiency and suggested potential undesirable effects (61; 62). Patients with sepsis treated with high doses of methylprednisolone showed an increased mortality two weeks after the start of therapy and their hemodynamic variables were unaffected (63). In the middle of 90s, two meta-analysis reversed the tide and serious doubts were cast on the efficiency of glucocorticoids in sepsis. The first study involved nine prospective randomized controlled studies and concluded that glucocorticoids did not have favorable effects on morbidity and mortality in severe sepsis (64). In the second meta-analysis, steroids appeared to increase mortality among patients with overwhelming infection and no effect was noted in the subgroup of patients with septic shock (65). When carefully examined, it is apparent that the literature had several dissonant reports, even when the paradigm of high dose of steroid for sepsis was the prevailing one. For instance, early clinical studies (66) using the limb perfusion technique have already shown that steroids failed to improve hemodynamic status during septic shock. Two well controlled studies with small numbers of patients in septic shock failed to show any evidence of beneficial effects of large doses of steroids (67; 68). These studies were criticized due to flaws in design and group size and soon disappeared into oblivion. The most likely reason of this oversight was that researchers were probably seduced by former positive results of steroids in sepsis and their successful use in other diffuse inflammatory process (69). The doubts raised by these two groups had to wait for the two big meta-analysis already mentioned to confirm their warnings. In summary, although initially used in high doses in sepsis for their anti-inflammatory properties, the mounting negative results and the two meta-analysis strongly discouraged further use of corticotherapy in patients with sepsis or septic shock. A consensus was reached indicating that pharmacological doses of steroids in sepsis and septic shock should not be used because they do not improve survival and may even increase morbidity and mortality (61; 63; 68; 70; 71). Therefore, the research on the use of glucocorticoids in sepsis was halted for almost a decade, until the concept of relative adrenal insufficiency in sepsis started to emerge.

4.3. Relative adrenal insufficiency in sepsis

It is now well recognized that an enhanced adrenal cortical secretion is present in severe infections. The steroid response in sepsis seems to be essential for survival. Adrenalectomy decreases the tolerance of animals to experimentally induced septic shock. For instance, sublethal doses of endotoxin or live bacteria for normal animals kill adrenalectomized rats (72; 73). Giving corticosteroids to adrenalectomized animals improves the outcome (72). Drugs suppressing adrenocortical function led to an increased mortality in critically ill patients (74). Therefore, cortisol may be a protective element in the homeostatic response to injury and normal adrenal function may be important for survival in sepsis. Interestingly, adrenocortical insufficiency in children with meningitis have been described some decades ago (75) and this finding has been associated with impaired cardiac performance (76). Thus, these reports were suggestive that some patients responded with rapid clinical improvement to glucocorticoid administration. These observations led to the hypothesis that adrenocortical insufficiency may occasionally exist in severe infections (77). Based on the protective effect of steroids, corticoid replacement therapy was considered for sepsis treatment. In a small trial, hydrocortisone was shown to improve dramatically survival rate of patients with presumed adrenal insufficiency (78). In the early 90s, it was reported that some patients with septic shock may have relative adrenocortical insufficiency and that low-dose hydrocortisone infusion in septic shock attenuated the systemic inflammatory response (79). Cortisol replacement therapy improved the hemodynamic condition in refractory septic shock (80). Subsequently, several experimental and clinical investigations have suggested that a reversible dysfunction of the HPA axis may occur during sepsis (81; 82). This line of evidence indicated that steroid use in sepsis should be reconsidered. However, this use should be directed to replacement therapy with low dosage rather than pharmacological (high) doses suggested by former studies.

Thus, whereas negative results were obtained with high doses of corticoids, studies performed with lower doses and for long periods showed promising results (81; 83; 84). The interest in this new approach increased further following two clinical studies, which showed that low doses of hydrocortisone restored vascular reactivity to vasoconstrictors in septic shock (85; 86). This beneficial effect was particularly important in patients with relative adrenal insufficiency.

In human septic shock, impaired adrenal function reserve is present in around 40% of patients (79). The importance of recognizing inadequate adrenal corticosteroid production has become more relevant over the last decade, as the use of exogenous glucocorticoids for sepsis and septic shock has decreased dramatically, whereas the use of drugs that can affect adrenal cortex function has increased (87). Sepsis may be a considered as a generalized imbalance of physiological processes. Thus, to bring the normal physiology back may be a better option than to combat aggressively the altered homeostasis. Within this background and since endogenous corticoids appear to have a protective effect in sepsis, a replacement therapy for patients with adrenal insufficiency sounded reasonable, making good use of the suggestion to perform

corticotrophin stimulation tests to assess the adrenocortical function in patients with septic shock (79). The beneficial effects of small doses of hydrocortisone were limited to the subgroup of patients with sub-normal adrenal response to corticotropin (88). However, testing HPA axis responsiveness is not a trivial issue and the use of adrenocortical stimulation tests in patients remains controversial (89; 90).

In any event, the low-dose steroid protocol could restore homeostasis and increase vasomotor tone without causing immunosuppression, one of the much feared steroid detrimental effect. Thus, the prolonged administration of modest doses of hydrocortisone within the newer paradigm of relative adrenal insufficiency in septic shock was a primary clinical interest until recently.

As described above, low doses of corticoids attenuated inflammation, restored vessel reactivity to vasoconstrictors, and improved survival. At least part of the beneficial effect of steroids in reducing the need of vasoconstrictors may be related to the resensitization of adrenergic receptors, which have desensitized during septic shock. This effect seems to involve both alpha and beta adrenergic receptors (83; 85; 86). The improvement in endogenous vasoconstrictors effects allows further reducing of the dosage of those drugs as well as their side effects (84). Interestingly, patients with normal to high cortisol levels did not benefit from corticoid therapy (85).

The use of low dose/long-term administration of hydrocortisone has not been proved to be effective and safe for all patients with septic shock. Additionally, it is very difficult to define adrenal insufficiency and to ascertain that restoration of hemodynamic status reflects correction of adrenal insufficiency. CORTICUS, an ongoing international, multicenter, randomized trial of corticosteroids in sepsis, should help to answer these important questions. Recently, a part of CORTICUS results were published (91). Data are not conclusive and further studies are still needed to optimize the diagnosis of adrenal insufficiency in critical illness. Due to the favorable initial small studies showing beneficial effect of low dose/long-term glucocorticoid regimen, current guidelines from Surviving Sepsis Campaign recommend low doses of corticoids in selected cases of sepsis/septic shock (92; 93).

5. NITRIC OXIDE AND STEROIDS IN SEPSIS

5.1. Introduction

There is no doubt on the central role played by NO in sepsis and septic shock. Although there are plenty of information on the consequences of NO production on the cardiovascular aspect of sepsis, far less information is available regarding the inflammatory, immune and cytotoxic aspects. In the next few pages, we will summarize the (yet small) literature concerning NO, sepsis and steroids.

5.2. Effects of NO on receptor binding and expression

It was observed a decrease of steroid binding in liver cytosol obtained from endotoxemic mice (94). One year later these authors showed that bacterial endotoxin dose-dependently reduced the binding of dexamethasone to cytosolic receptor in the liver and that this effect was associated with an impairment of glucocorticoid-induced increase in hepatic enzymes (95). A generalized loss in GR binding has been observed in a variety of tissues including liver, kidney, skeletal muscle, spleen, lung and heart following administration of endotoxin (96). Soon it was reported for the first time a clinical evidence of this effect, by showing that GR binding was decreased in patients with septic shock. Interestingly, levels of plasma cortisol in these patients were higher than of normal volunteers, suggesting that the defect was not in cortisol secretion (97). These authors suggested that the marked decrease of GR might be the source of GC failure in sepsis and that massive doses of GC should be used at early sepsis when GR levels have not yet decreased. Thus, these reports suggested that diminished corticoid effects in sepsis was not caused by adrenocortical insufficiency but by reduced receptor binding capacity. Glucocorticoid hypofunction caused by reduced receptor binding capacity may accelerate tissue injury (98; 99).

Mechanisms leading to loss in steroid function remain to be described. A factor from peritoneal macrophages that interfered with the inductive action of glucocorticoid was described and named glucocorticoid antagonizing factor (100; 101). Later it was described that the altered glucocorticoid action during endotoxic shock was mediated by a factor(s) other than endotoxin and the likely source of this mediator(s) was the macrophage (102). Some months later, a new research field (apparently unrelated to steroids and their receptors) was inaugurated with the discovery of the chemical identity of nitric oxide (103) and its enzymatic synthesis (104). Quickly it has become apparent that NO contributes to several key features of septic shock physiopathology, such as severe arterial hypotension, vascular hyporeactivity to vasoconstrictors and myocardial dysfunction (reviewed in 105). The first report directly linking GR and NO was published in 1999 (106). The authors described that NO donors decreased in a time- and dose-dependent manner hormone binding to GR. Scatchard plots revealed that the number of ligand binding sites were reduced by NO donors, with a discreet effect on GR affinity. Western blotting analysis showed that NO did not change GR protein levels. The authors suggested that nitrosation of one or more critical cysteine residues on GR may be the mechanism of GR decreased binding. These findings were later extended and confirmed in an *in vivo* model, using bacterial endotoxin as a surrogate model of sepsis (107) and using cecal ligation and puncture (Duma et al., unpublished results) model, which best reproduces the human condition. In both models, inhibition of NOS completely prevented GR failure, indicating that NO is indeed a key factor in GR malfunction in sepsis. Taken together these results indicated that glucocorticoid failure to induce their potent anti-inflammatory effects in animals exposed to NO (either by NO donor injection or LPS injection) may be explained, at least in part, by NO-induced inhibition of glucocorticoid receptor binding.

The issue of NO effects on GR is far from being resolved. Indeed, it has been shown that GR tyrosine nitration leads to the enhancement of GR-mediated events (108). However, it should be noted that the authors have used a nitrosteroid, which release low NO amounts in a closed microenvironment. One possible explanation for this contrasting results would be that, as found in other contexts, NO concentration is the key factor in controlling the final result. Since in sepsis/septic shock high NO levels are present, specially at the height of this condition, may be this facilitatory effect does not take place. In any event, further studies should be performed with these compounds, as they may be good therapeutic options to treat sepsis.

Studies regarding the effects of inflammatory stimulus on GR expression have yielded mixed results. In fact, although the majority of studies demonstrated reduced GR function and/or reduced GR affinity, in those studies that additionally examined GR expression, some found down-regulation (98; 109-111) whereas others found up-regulation (112-115). These discrepancies may result from the use of different types of cells and tissues, as well as different treatments.

There are few studies on GR expression in *in vivo* models or patients with sepsis or septic shock. In tissues from animals bearing chronic sepsis it was found a decrease of 30% in the steady-state level of GR mRNA (116). Peripheral blood mononuclear leukocytes from patients with sepsis or septic shock showed a down-regulated GR (111). More recently, it has been shown that endotoxin infusion in a porcine model markedly reduces GR expression in lung, liver and kidney (117). Altogether these data suggests that a reduction on GR expression, and not only changes in their properties, may be responsible to loss in GR functionality during sepsis. More studies are required to clarify this point.

To add another layer of complexity in the triangle NO/GR/sepsis, it was demonstrated that inhaled NO could stimulate glucocorticoid receptor up-regulation in non-pulmonary tissues, thereby increasing the effectiveness of therapeutic doses of glucocorticoids (117). Numerous studies have debated the role of NO in different models of shock, and several investigators have even demonstrated beneficial effects of NO (118). For example, NO may exhibit protective effects in systemic inflammatory conditions, such as by improving organ blood flow (119), inhibiting platelet aggregation (120), and reducing plasma concentrations of pro-inflammatory cytokines (121). Inhaled NO has been recently shown to have extra-pulmonary effects (122) and would provide protective effects on septic cardiovascular dysfunction (123; 124). The very nice study by Da and colleagues (117), has shown that the anti-inflammatory mechanism of inhaled NO is, at least in part, consequence of increased in GR protein and that the association of inhaled NO and steroids would make steroid therapy more effective. However, a cautionary note must be added since treatment (steroid and/or inhaled NO) was made at early times after LPS infusion (2.5 h), when probably NO production by NOS-2 has not yet took place.

Finally, NO donors can directly activate GR present in the endothelium (125). Since glucocorticoids regulate the expression of their own receptors (126-128), some of effects of NO on GR expression may be the consequence of direct activation of GR by NO. In the same line of thought, one may wonder if the putative NO direct effect on GR may represent a potential feedback mechanism to regulate endothelial NOS-2 expression. Interestingly, all reports summarized here have looked on NO effects on GR binding or expression, but none have examined direct effects of NO on GR activation.

5.3. Glucocorticoids versus mineralocorticoids in sepsis

From what was discussed so far, it became clear that during sepsis there is an impairment in glucocorticoid functionality and that the high NO production appear to be directly involved in this condition. These data are in agreement with convincing evidence that corticosteroids administered in high doses during sepsis are not effective, and might even be harmful. However, an still open and intriguing question remain: if glucocorticoid receptors are not functional during sepsis, how low-doses of glucocorticoids work? In same line of thought, what is the relevance (if any) of mineralocorticoids for sepsis?

Mineralocorticoid receptors have a higher affinity for glucocorticoids than GR itself and glucocorticoids bind to GR with lower affinity when compared with mineralocorticoids. However in normal conditions, GR is expressed in much higher density and the effects of this receptor are more pronounced (129). Interestingly, fishes have both receptors (MR and GR), although they do not possess aldosterone or the enzymes that produce it (130), suggesting that MR originally may have served as a high affinity glucocorticoid receptor. Although highly speculative at this moment, it seems logical to suppose that positive effects of glucocorticoids in sepsis may be, at least in part, mediated by mineralocorticoid receptor.

In agreement with this hypothesis, animal studies have shown that aldosterone inhibited interleukin-1-induced nitrite synthesis in vascular tissues (131). Additionally, it was reported that aldosterone enhanced the vasoconstrictor response to phenylephrine in large mesenteric vessels (132). This effect was significantly reduced by eplerenone (MR receptor antagonist), suggesting that the canonical MR was involved. Another evidence is that in critically ill patients, there is an inhibition of aldosterone secretion (133), which are associated with the lack of renal sodium and water reabsorption followed by increased development of acute renal failure. Although scanty, these data has lead to the proposal that mineralocorticoid substitution may be relevant in septic shock and corticoids like dexamethasone, which has no mineralocorticoid activity, should be discouraged to treat sepsis (44). Finally, a word of caution. Aldosterone exerts many harmful effects in the cardiovascular system in addition to its important physiologic role in electrolyte and water homeostasis. These adverse effects include endothelial dysfunction, cardiomyocyte apoptosis, induction of vascular inflammation, myocardial ischemia and necrosis, reduced

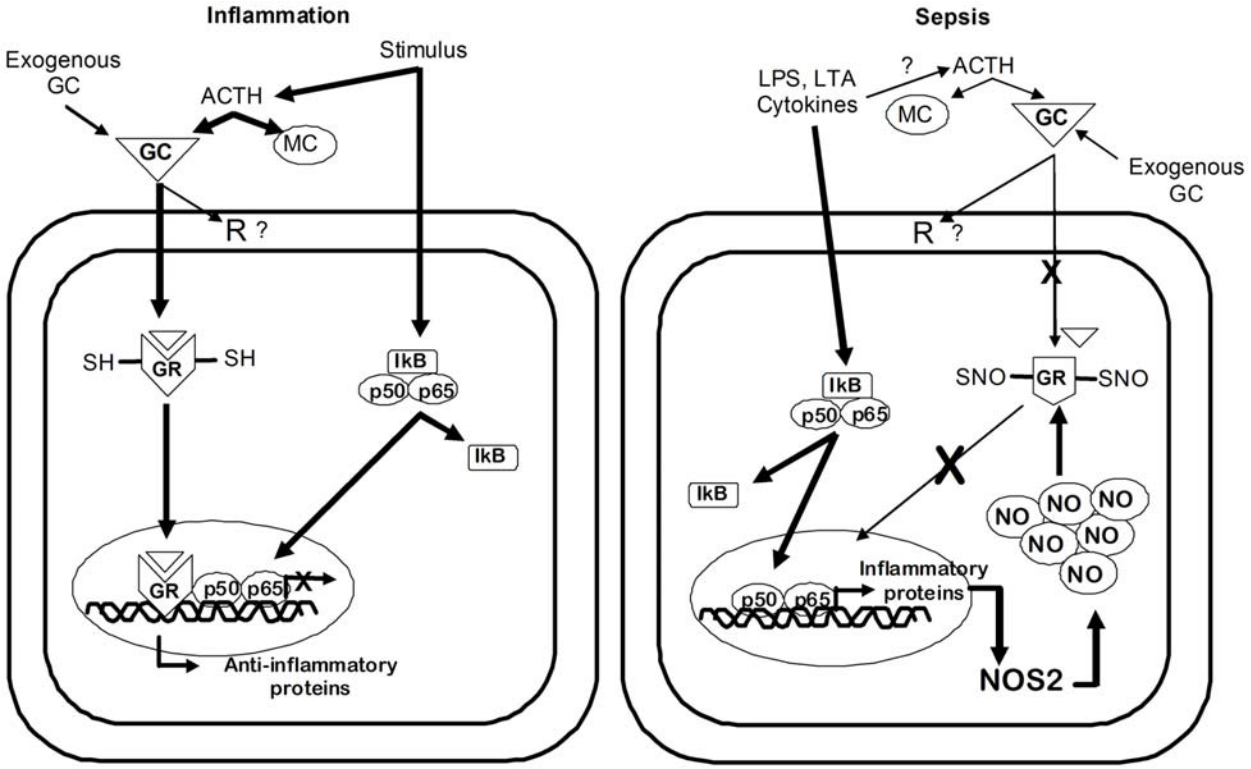

Figure 2. Upon inflammatory stimulus, steroids (GC and MC) are released from adrenal cortex by ACTH. GC may interact with putative membrane receptors or with the canonical cytosolic GR. Upon GC binding, GR shed its companion proteins and the free form translocates to cell nucleus, dimerize, bind to accessory proteins and repress/activate specific genes. Inflammatory stimulus activate NF-kB which after dissociation of I-kB, translocates to cell nucleus. Transcription activity of NF-kB may be blocked by GR. In sepsis, endotoxins, exotoxins and cytokines induce NF-kB activity and expression of pro-inflammatory proteins. ACTH may be at lower concentration or activity which would release lower steroids concentrations. In addition, increased expression of NOS-2 produces high NO concentrations which may nitrosate GR sulphydryls, reducing its binding capacity and biological activity. For the sake of clarity only GR pathway is detailed. Thick arrows indicate higher activity or preferential pathway. Double-crossed arrows mean inhibition or reduction. Interrogation marks mean unknown mechanism or not definitely demonstrated mechanism. Abbreviations: ACTH, adrenocorticotropic hormone; GC, glucocorticoid; MC, mineralocorticoid; R, receptor; -SH, cysteine sulphydryls; GR-SNO, nitrosated GR; I-kB, NF-kB inhibitor; LPS, lipopolysaccharide; LTA, lipotheicoic acid; NO, nitric oxide; NOS2, inducible NO synthase.

arterial compliance and decreased baroreceptor sensitivity (for review see 134). In summary, if the relationship between NO, GR and sepsis is still a matter of debate, the understanding of the involvement of mineralocorticoids in sepsis has yet a long road to be walked on.

6. CONCLUDING REMARKS

The ideas presented in Figure 2 summarize some of the current concepts on steroids, NO and sepsis. Hopefully it will provide to the interested reader a conceptual framework of knowledge that has to be gathered in order to deal with the still open questions presented below.

Steroids are important compounds not only in physiology but in pharmacology. In spite of their plethoric effects, their powerful anti-inflammatory properties make them valuable tools for treat conditions when other therapeutic classes (such as non-steroid anti-inflammatory or disease-modifying drugs) have failed. This is particularly true for chronic inflammatory conditions, when glucocorticoids are the last resort. Although sepsis and septic shock are clearly high-intensity inflammatory conditions it is both frustrating and puzzling that steroids, in special glucocorticoids, fail so miserably. Even if the present clinical trials confirm that low dose/long-term regimen is useful in sepsis, patients which would benefit from it represent only a modest fraction of the large numbers of sepsis/septic shock-induced deaths around the world. However, looking from a more optimistic angle, these big gaps in our knowledge may hide hitherto valuable information on how better use steroids in sepsis. As a contribution to the field, it is possible to point some avenues that may prove fruitful in the future. First, we need a better understanding of the mechanisms that preclude GC of working properly in sepsis. Second, we need to know what is the role played by mineralocorticoids in sepsis. Third, we need to have a clear comprehension of the meaning of glucocorticoid insufficiency in sepsis and its

consequences for the progression of the condition. Fourth, it is important to understand how the balance between "desirable" (anti-inflammatory) and "undesirable" (immunosuppressive) effects will affect sepsis outcome. Surely, we still have a long road to walk. But a very exciting one.

7. ACKNOWLEDGMENTS

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