Glucocorticoids –
New Mechanisms
for Old Drugs

Glucocorticoid (GCC) therapy affects a wide range of cells and tissues and produces a spectrum of effects that vary with drug concentration and cell type. Labeled indications for GCC use in a variety of species may be broadly stated as “antiinflammatory” or the label may list specific indications for use.

In a 2004 monograph prepared by the United States Pharmacopeia Veterinary Medicine Expert Committee on Drug Information, indications for drugs were grouped into three categories: clear evidence to support use of a drug for a particular purpose (accepted); scant evidence or evidence subject to concern based on experimental design (acceptance not established); or lack of evidence of effectiveness (unaccepted). Categorizations were applied to both label and extralabel uses.¹

Indications for GCC therapy categorized as “accepted” include adrenocortical insufficiency, allergic disorders including allergic dermatitis, nonallergic dermatoses, asthma, otitis, pruritus, musculoskeletal inflammation, ocular inflammation in the absence of corneal ulceration, adjunct therapy of septic shock (endotoxemia), immune-mediated hemolytic anemia and immune-mediated thrombocytopenia, diagnosis of hyperadrenocorticism, systemic lupus, lymphoma, mast cell tumor, pemphigoid and pemphigus diseases, selected neurological disorders, selected cases of ulcerative colitis, induction of abortion in cattle, and bovine ketosis.¹ These many and varied

Veterinary Indications for GCC Use:

- Adrenocortical insufficiency
- Allergic disorders including allergic dermatitis
- Nonallergic dermatoses
- Asthma
- Otitis
- Pruritus
- Musculoskeletal inflammation
- Ocular inflammation in the absence of corneal ulceration
- Adjunct therapy of septic shock (endotoxemia)
- Immune-mediated hemolytic anemia and immune-mediated thrombocytopenia
- Diagnosis of hyperadrenocorticism
- Systemic lupus
- Lymphoma
- Mast cell tumor
- Pemphigoid and pemphigus diseases
- Selected neurological disorders
- Selected cases of ulcerative colitis
- Induction of abortion in cattle
- Bovine ketosis
therapeutic uses of GCC are mediated by drug binding to the glucocorticoid receptor (GR) with subsequent effects on multiple signaling pathways.

The purpose of this discussion is to review previously described as well as newly elucidated molecular mechanisms of GCC action and to relate these to developments in drug therapy.2,3

MECHANISMS OF ACTION

The hypothalamic-pituitary-adrenal axis controls secretion of cortisol, the primary endogenous GCC. Neural, endocrine, and cytokine signals influence the hypothalamus and trigger release of corticotropin-releasing hormone (CRH) that in turn prompts release of adrenocorticotropic hormone (ACTH) from the anterior pituitary. ACTH then stimulates synthesis and secretion of cortisol from the adrenal cortex. Greater than 90% of cortisol is bound to corticosteroid-binding globulins, leaving the remaining free, biologically active cortisol to be converted to cortisone by type 2,11-β-hydroxysteroid dehydrogenase. The effects of cortisol and exogenously administered GCCs rely on several molecular mechanisms. Three are described below.

Direct Effects of GCC on Gene Expression2

Cortisol or exogenously administered GCCs bind to the GR, a member of the steroid hormone receptor superfamily that includes, among others, the mineralocorticoid, estrogen, and progesterone receptors. A frequent feature of the steroid hormone-signaling pathway is the presence of a chaperone, or heat shock, protein that binds to the unoccupied receptor and renders it inactive. Binding of ligand to receptor promotes dissociation of the heat shock protein and translocation of the activated ligand-receptor complex to the nucleus. This process is followed by binding of a dimerized ligand-receptor complex to a GCC response element (GRE) in the DNA. A GRE is comprised of a consensus sequence located in the regulatory promoter region of a GCC-responsive gene. A given gene may have multiple GREs and, when bound by activated ligand-receptor complexes, other proteins join the complex to either promote or repress gene transcription via RNA polymerase II (Figure 1).

Examples of gene products that are induced by GCCs include annexin I (lipocortin 1) and MAPK phosphatase I. Both of these products inhibit a key proinflammatory pathway triggered by the activation of cytoplasmic phospholipase A2α, which prompts the release of arachidonic acid from membrane phospholipids, leading to the production of prostaglandins and leukotrienes. GCC-mediated inhibition of this enzyme thus decreases the production of these proinflammatory mediators (Figure 2).

MAPK phosphatase I also inhibits another important proinflammatory pathway mediated by a complex referred to as activator protein 1 (AP-1). AP-1 may be a heterodimeric complex composed of two proteins, c-Jun and Fos, or a homodimer of c-Jun. When phosphorylat-
ed, AP-1 binds to AP-1 DNA response elements and induces transcription of inflammatory and immune genes. When GCCs induce MAPK phosphatase I, this enzyme indirectly decreases activation of AP-1 by limiting phosphorylation of the complex. Direct genomic effects of GCC can also cause decreased transcription of genes with a reduction in gene product; e.g., GCCs repress transcription of cyclooxygenase 2, an enzyme responsible for production of proinflammatory prostaglandins.

The direct genomic effects of GCCs have traditionally been cited as the primary mechanism for GCC action.

**Interference with Transcriptionally Active Proteins**

It is now clear that other arms of the GCC-signaling pathway also play important roles in the actions of these drugs. Specifically, interaction of the cortisol-GR complex with other inducible transcription factors alters...
expression of genes responsive to these transcription factors. Nuclear factor-kappa B (NF-κB) signaling is of prime importance to the understanding of the antiinflammatory effects of GCC and the effects of other agents including nonsteroidal antiinflammatory drugs (NSAIDs). Inhibition of NF-κB by GCC is thought to occur at lower cortisol levels than those needed to affect transcription through binding of activated GR to GREs.

NF-κB plays an important and highly conserved role in coordinating the expression of leukocyte adhesion molecules and soluble proinflammatory mediators such as cytokines and chemokines. NF-κB in an unstimulated cell is sequestered in the cytoplasm by an inhibitory protein referred to as inhibitor of NF-κB (IκB). When bound to IκB the nuclear localization signal on NF-κB is masked, preventing movement of NF-κB into the nucleus and precluding its actions as a proinflammatory transcription factor.

Upon stimulation of a cell by proinflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-1 (IL-1) or with bacterial lipopolysaccharide (LPS), a specific IκB kinase complex is formed that phosphorylates IκB, thereby tagging it for ubiquitination and degradation. Degradation of IκB allows NF-κB to move to the nucleus and assume its role as an active transcription factor. Many genes involved in inflammation, e.g., cytokines, chemokines, cell-adhesion molecules and complement factors, contain NF-κB binding sites within their promoters and are thus responsive to NF-κB binding. NF-κB also induces the transcription of cyclooxygenase 2 and enhances prostaglandin synthesis (Figures 1 and 2).

GCCs are thought to inhibit NF-κB activity through a variety of mechanisms including increased expression of an IκB molecule and physical interaction between NF-κB and the GCC-occupied GR in the nucleus. Activated GR also interacts with and directly inhibits other proinflammatory transcription factors such as AP-1.

**Nongenomic Mechanisms**

Mechanisms that do not rely on changes in gene transcription, i.e., nongenomic mechanisms, are now thought to play a role in GCCs rapid inhibitory actions on inflammation. An example of a nongenomic mechanism is activation of endothelial nitric oxide synthetase, resulting in production of nitric oxide. Increased nitric oxide protects against ischemia or reperfusion-related injuries. Other nongenomic actions of GCC include decreasing the stability of messenger RNA products of inflammatory genes such as vascular endothelial growth factor and cyclooxygenase 2 (Figure 1).

**Clinical Relevance of GR Structure-Function Relationships**

Our understanding of molecular mechanisms of GCC antiinflammatory action has advanced, but this knowledge has not yet resulted in improved drugs with fewer side effects. Decreasing the undesirable effects of GCCs will likely depend upon a better understanding of structure-function relationships between GCC ligands and different GR isoforms. Some experts suggest that antiinflammatory effects of GCC are mediated primarily by inhibition of NF-κB and AP-1 and that side effects are more attributable to GR mediation of transcriptional events. If these two mechanisms could be differentially activated, more targeted therapeutic interventions could result.

Variation in the structure and expression of GR isoforms results in diversity in GCC signaling. In the human GR gene and gene product, alternative sites for the initiation of transcription in exon 1, alternative splicing of premessenger RNA, alternative translation initiation sites, and varied posttranslational modification all contribute to diversity in functional expression of the receptor.

The α isoform of the human GR binds cortisol, DNA, and other transcription factors and may also act through nongenomic pathways. The β isoform has no known transcriptional activity but can form heterodimers with the α isoform that may interfere with activity of this protein. It is thought that the ratio of α to β isoforms may serve as a regulatory mechanism that could dictate the relative sensitivity or resistance of a cell to GCC action. Higher levels of β isoforms would be expected to correlate with GCC resistance.

Additional human GR isoforms, designated A and B,
result from differential translation start sites and lead to functionally different isoforms. GR α-B is known to have a greater biological activity than GR α-A and the two are found in different ratios in different cells and tissues.

The potential for functional GR heterogeneity is multiplied by the diversity of species treated in veterinary medicine. Understanding the molecular structure and functional relationships of GR isoforms may lead to development of drugs that activate selected mechanistic pathways with enhanced antiinflammatory properties and diminished side effects.

**Future Directions**

Improved understanding of NF-κB signaling has revealed roles in the stress response, apoptosis, cell survival, oncogenesis, and development. Cell type and differentiation state appear to have major effects on the outcomes of the pathway in a given cell type. NF-κB is now known to play a central role in skin, including balancing growth and differentiation in the epidermis (Figure 3). The model for NF-κB signaling in the epidermis is based on a classical role for NF-κB in cell survival but an atypical role for this factor in promoting cell cycle withdrawal. NF-κB appears to be activated when basal epidermal cells withdraw from the cell cycle and commit to terminally differentiate. The convergence of cell survival and cell cycle arrest pathways, linked through NF-κB, may help to balance homeostasis in the epidermis. Understanding the role of NF-κB in skin will provide a foundation for designing and assessing new therapeutic approaches that manipulate the NF-κB pathway.

The realization that GCCs, as well as other commonly used drugs such as NSAIDs, act in part by a NF-κB-mediated mechanism has made this a vital area of investigation. As the sequence of events in the proinflammatory NF-κB pathway has been elucidated, a variety of new and old drugs have been classified according to their ability to inhibit selected steps. NF-κB inhibitors that demonstrate antiinflammatory activity in experimental models include salicylates, other NSAIDs, GCC, antisense oligodeoxynucleotides, transcription factor decoys, antioxidants, proteasome inhibitors, peptides, and natural compounds including flavanoids and polyphenols.

**REFERENCES**