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Corticosteroids and perinatal hypoxic-ischemic brain injury

Katherine R. Concepcion¹ and Lubo Zhang¹

¹Lawrence D. Longo, MD Center for Perinatal Biology, Department of Basic Sciences, Loma Linda University School of Medicine, Loma Linda, CA 92350, USA

Abstract

Perinatal hypoxic-ischemic (HI) brain injury is the major cause of neonatal mortality and severe long-term neurological morbidity. Yet, the effective therapeutic interventions currently available are extremely limited. Corticosteroids act on both mineralocorticoid (MR) and glucocorticoid (GR) receptors and modulate inflammation and apoptosis in the brain. Neuroinflammatory response to acute cerebral HI is a major contributor to the pathophysiology of perinatal brain injury. Here, we give an overview of current knowledge of corticosteroid-mediated modulations of inflammation and apoptosis in the neonatal brain, focusing on key regulatory cells of the innate and adaptive immune response. In addition, we provide new insights into targets of MR and GR in potential therapeutic strategies that could be beneficial for the treatment of infants with HI brain injury.

Keywords

corticosteroid; dexamethasone; glucocorticoid receptor; mineralocorticoid receptor; encephalopathy; inflammation; microglial; astrocyte; cytokine; chemokine; apoptosis; neonatal brain damage; HI; HI; hypoxia; stroke; neuroprotection

Introduction

Perinatal HI encephalopathy (HIE), a subgroup of neonatal encephalopathy, occurs in approximately three per 1000 live births and remains the leading cause of death in term infants [1,2]. HIE is most commonly caused by an intrapartum HI insult, such as placental abruption, uterine rupture, and umbilical cord accidents. The morbidity and mortality of HIE is 23% with clinical consequences of seizures, cerebral palsy (CP), visual and hearing impairments, and mental abnormalities [1,3,4]. The clinical indications for HIE-induced CP are an umbilical cord pH less than 7.0, evidence of moderate-severe neonatal encephalopathy, and absence of other CP causes [1]. These associated comorbidities require significant, costly medical assistance throughout life and negatively affect the quality of life of the patient and their productivity as an adult [3]. Hypothermia, the standard of care for

Corresponding author: Concepcion, K.R. (kconcepcion@llu.edu).

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treatment of HIE, targets the cerebral metabolism and key injury mechanisms that occur in HIE [5]. Hypothermia, when started within 6 h of birth, significantly reduces the morbidity and mortality of newborns with HIE, yet many infants (>40%) die or experience severe neurological deficits after treatment [6]. In a multicenter, randomized trial of hypothermia treatment for patients with HIE, there was still an abrupt increase in proinflammatory cytokines despite hypothermia treatment [7]. It is clear that adjuvant therapies are needed to alleviate the severity of HIE outcomes and comorbidities.

The pathogenesis of HIE is complex, involving short-term neuronal damage that evolves into long-term chronic inflammation. The mechanisms responsible for the progression of HI brain injury include: excito-oxidative-, free-oxygen-radical-, caspase-, and cytokine-mediated cell damage, interrupted calcium and mitochondrial homeostasis, and inflammatory cell activation and recruitment [8,9]. Early events of HIE are dominated by damaged neuronal cells via the excito-oxidative cascade. During initial HI insult, ATP depletion occurs rapidly [10], leading to Na^+/K^+ -ATPase pump failure and depolarization of the cells, resulting in calcium accumulation through reversal of the $\text{Na}^+/\text{Ca}^{2+}$ -exchange carrier. Subsequent influx of calcium occurs, causing cellular swelling and irreversible neuronal energy failure, which ultimately leads to necrosis and a plethora of signaling cascades leading to more cellular death [11].

In HI brain injury, there is an early- and late-phase inflammatory process. The early inflammatory response lasts from hours to days. It is initiated by activation of microglial cells, the resident innate immune cells in the brain, by injured neuronal cells that release endogenous molecules and the proinflammatory cytokines $\text{TNF-}\alpha$ and $\text{IL-1}\beta$ [12]. Activated microglial cells subsequently release proinflammatory cytokines and proteases, and activate NMDA-mediated toxicity, which leads to secondary neuronal injury [9]. Astrocytes, the largest population of glia cells in the neonatal brain, have a protective role through glutamate uptake metabolism, maintenance of the blood-brain barrier (BBB), and glial scar formation after injury [13]. In HI damage, astrocytes generate extracellular glutamate dysregulation, neuronal axonal injury, and release of $\text{TNF-}\alpha$ and IL-6 [9]. Neutrophils first appear in the cerebral vasculature 4 h after HI injury in the neonatal rat brain and extravasate into the brain parenchyma 42 h after initial injury [9]. Depletion of neutrophils confers neuroprotection only when depleted before HI brain injury [14]. Lastly, T and B cells are implicated in the delayed neuroinflammatory response to HI injury, and persist in the long-term inflammatory response, up to 35 days after HI injury [9].

Recent studies reported that direct glucocorticoid administration to the neonatal brain via intracerebroventricular (i.c.v.) injection as well as intranasal administration provided neuroprotection and ameliorated brain damage in neonatal HI injury [15,16]. Other studies have also proposed glucocorticoids to have therapeutic potential for the treatment of HI injury, yet evidence of both neuroprotective and neurotoxic effects of glucocorticoids exists [17,18]. Several lines of evidence indicate that the timing, dosing, duration of treatment, and severity of disease influences the effects of glucocorticoids [18]. The long-term consequences of dexamethasone are controversial, yet it might be the best adjuvant option with hypothermia treatment in devastating inflammatory diseases, such as HIE or neonatal

stroke. Glucocorticoid agonists could have the potential to decrease inflammatory cytokines that negatively impact key neuronal cells.

Regulation of corticosteroid receptors in the developing brain

Mineralocorticoid and glucocorticoid receptors in the developing brain—GRs and MRs are crucial for fetal brain development and stimulation of the hypothalamic-pituitary-adrenal (HPA) axis for parturition, organ maturation, and fetal growth [19]. Their expression is region specific, occurring in the hippocampus and limbic system [19]. Throughout the fetal development, expression of GRs and MRs dynamically changes, giving insight into their function and control of the HPA axis (Figure 1).

In the mouse hippocampus, MR mRNA expression is first detected embryonically, whereas that of GRs is first expressed after birth [20,21]. Conversely, rat and guinea pig show detectable mRNA levels of GRs and MRs before birth, with increased levels of GR mRNA closer to birth [22–24]. During hippocampal formation during midgestation, when fetal cortisol levels are low, hypothalamic MR mRNA expression is more abundant than is GR mRNA expression [20,23–25]. Closer to birth, the progression of MR and GR mRNA switches. MR mRNA gradually decreases in the CA1/2 hippocampal region from midgestation to birth, whereas GR mRNA increases in the CA1/2 hippocampal region [24]. Notably, the expression of both human hippocampal MRs and GRs is detectable between 24 and 34 weeks of gestation [20]. No other time points in the human hippocampus have been studied. The molecular modifications of MRs and GRs studied in the hippocampus of the mouse, rat, and guinea pig hippocampus are best contextualized when evaluating the control of the HPA axis.

In the fetus, negative feedback on the HPA axis is decreased to allow for a high output of cortisol, which is required for maturation of organs, including lung, kidney, and brain [19,23]. This is made possible by several separate mechanisms. First, MR expression is thought to be mainly involved in HPA axis negative feedback control, and decreased MR expression closer to birth might release the HPA axis from this inhibition [24]. At the same time, GR expression is decreased in the paraventricular nucleus (PVN) of the hypothalamus, allowing for a further decrease in the negative feedback control [23,26]. In addition, levels of the enzyme 11 β -hydroxysteroid dehydrogenase type 2 (11 β -HSD2) fall to allow for high levels of adrenocorticotropin hormone (ACTH), corticotropin releasing hormone (CRH), and cortisol [27], 11 β -HSD2 converts cortisol to inactive cortisone to protect the fetus from glucocorticoid overexposure before the third trimester of gestation [27,28]. This results in high ACTH despite increased cortisol, allowing for a cortisol surge that is necessary for organ maturation.

Hypoxia and effects on MR and GR expression—Glucocorticoids are steroid hormones secreted by the adrenal gland and have an important role in the regulation of metabolism and immune response regulation. Endogenous glucocorticoids, such as cortisol, bind to MRs and GRs [29]. MRs have a relatively high affinity and are highly bound at basal levels of glucocorticoids, whereas GRs bind corticosteroids relatively weakly at physiological levels. Synthetic glucocorticoids, such as betamethasone and dexamethasone,

selectively bind GRs, but not MRs. GRs are retained in the cytoplasm through association with heat-shock protein 90 (HSP90) [30]. Once the ligand binds to the GR, HSP90 dissociates, and the activated GR–glucocorticoid complex is translocated to the nucleus.

Upon nuclear localization, the GR–glucocorticoid complex controls inflammation by several different mechanisms. The GR forms a homodimer at a consensus DNA site, called glucocorticoid response elements (GRE), where it recruits basal transcription factors, including SRC-1, TIF-2, p300/CBP co-integrator protein, and GRIP-1, to induce histone modifications and direct gene transcription [31]. The GR represses transcription of proinflammatory cytokine and chemokine genes through histone deacetylation of the key proinflammatory transcription factors nuclear factor kappa B (NF- κ B) and activator protein 1 (AP-1), thus preventing IL-8 and IL-1 β induction [31]. In addition, the GR activates the anti-inflammatory genes *IL10*, *IL1R2* (decoy receptor and IL-1 receptor antagonist), and the gene encoding secretory leukocyte inhibitory protein [30]. Furthermore, glucocorticoids induce histone deacetylase (HDAC) expression to inhibit CBP-associated histone acetyltransferase (HAT) activity, which is required for IL-1 β stimulated histone acetylation

Increasing evidence establishes a close linkage between hypoxia and glucocorticoids. In an acute hypoxic event during late gestation, plasma ACTH and cortisol levels increase transiently up to 7 days in the ovine fetus [34], indicating activation of the anterior pituitary-adrenocortical axis during late gestation. At term, neonatal hypoxia causes an increase in plasma ACTH and glucocorticoid levels [35]. One study in the neonatal pig showed better neurological outcomes in correlation with higher serum cortisol concentrations [36]. Interestingly, neonatal hypoxia caused long-term reprogramming through an augmented ACTH and corticosterone stress response later in the adult rat [37].

HIF-1 α , the master transcription regulator of the adaptive response to hypoxia, causes adaptive upregulation of GRs. This evidence revealed that the GR α promoter region contains five putative HIF-1-binding hypoxia response element (HRE) sites [32]. As a result, hypoxia enhanced dexamethasone-mediated inhibition of the NF- κ B proinflammatory mediators IL-1 β and IL-8 in an *in vitro* model [32]. Conversely, there is evidence that hypoxia also induces the opposite result, possibly because of a context-specific effect in the microenvironment of *in vivo* models. Chronic fetal hypoxia downregulates GRs through increasing DNA methylation of the GR promoter, decreasing binding of the transcription factors Egr-1 and SP1, and thereby increasing the vulnerability of the brain to hypoxia after birth [16]. Furthermore, in neonatal HI injury, inhibition of HIF-1 α after HI insult is neuroprotective by preserving BBB integrity and reducing brain edema [38]. Conversely, in severe neonatal HI models, HIF-1 α induction attenuates BBB permeability through vascular endothelial growth factor (VEGF) inhibition [39]. It is likely that GR and HIF-1 α co-localize in the nucleus and influence the adaptive response to HI insult, causing metabolic, apoptotic, and inflammatory differences.

Effects of the MR and GR on brain inflammation: neonatal models

Apoptosis

Neuroprotective mechanism of glucocorticoids: Primary energy failure is the first sign of damage in neonatal brain after HI injury, characterized by decreases in ATP and glucose, with increases in extracellular glutamate and intracellular calcium [9]. Studies by Tuor *et al.* provide evidence that dexamethasone-mediated alterations in metabolism protect against neonatal ischemic damage in the neonatal brain [40,41], although studies in the adult brain and chronic stress argue the opposite [42]. In both models, dexamethasone preserved levels of high-energy phosphates, such as ATP and phosphocreatine, and increased the glycolytic flux [40,42]. In the neonatal model, increased glycolytic flux appears to be an adaptive response by proper maintenance of ATP demand. This mechanism provides an explanation for neonatal resistance to cellular swelling and excitotoxicity through maintenance of the Na⁺/K⁺-ATPase pump. The Na⁺/K⁺-ATPase pump acts via hydrolysis supplied by free energy from ATP utilization. Conservation of Na⁺/K⁺-ATPase pump activity provides a proper sodium gradient that allows for Na⁺/Ca²⁺-exchange carrier activity and resistance of calcium accumulation, cellular swelling, and necrosis seen during the initial phases of ischemic neuronal damage [12].

Recent evidence revealed a secondary mechanism of neonatal adaptation to cytosolic calcium accumulation. Cytosolic calcium accumulation is further resisted by dexamethasone-mediated plasmalemmal Ca²⁺ ATPase activation in neonatal neurons and astrocytes [43]. Without this adaptive mechanism, subsequent cellular swelling and necrosis would occur, triggering the inflammatory response of the host caused by release of cellular contents into the extracellular space [44].

In addition, mitochondrial dysfunction through exacerbation of hypoxia-induced ATP loss in hippocampal astrocytes gives an initial mechanism of HI-induced damage in the neonatal brain [45]. The intrinsic pathway of apoptosis is activated in HI injury, causing mitochondrial permeabilization, release of proapoptotic proteins (cytochrome c and apoptosis-inducing factor), and subsequent caspase activation [44]. One way to resist apoptosis is to impede mitochondrial permeabilization. To stabilize the mitochondrial membrane and provide a neuroprotective effect, Bcl-xL proteins are upregulated and Bax proteins are downregulated [46,47]. Interestingly, pretreatment with glucocorticoids does not appear to act via a Bcl-2 mechanism [48]. Glucocorticoids appear to exacerbate hypoxia-induced upregulation of proapoptotic protein Bnip3, which is a member of the Bcl-2 family [49]. By contrast, the extrinsic pathway of apoptosis is mediated by death receptors, some of which are linked to the proinflammatory cytokines TNF- α and IL-1 β . TNF- α activity is mediated by TNFR1 and TNFR2, causing downstream apoptosis. Dexamethasone potently inhibits TNF production, providing neuroprotection in the adult rat exposed to permanent middle cerebral artery occlusion (MCAo) [50]. Additionally, treatment with corticosterone provides significant reductions in TNF- α production, attenuating the inflammatory-mediated response in neonatal HI-injury [51]. Both extrinsic and intrinsic apoptotic pathways converge on cleavage and activation of caspase-3. Of importance, caspase-3 activity peaks 24 h after HI-induction, and caspase inhibition elicits neuroprotection in neonatal rats [8]. Furthermore, glucocorticoids provide neuroprotection by inhibiting cleaved caspase-3 [52].

Through this mechanism, glucocorticoids are thought to affect downstream apoptotic pathways and decrease neuronal cell death in neonatal brain injury (Figure 2).

Another mechanism by which glucocorticoids protect neuronal cells from cell death is by acting on prosurvival signals. PI3K/Akt is a key pathway that mediates neuronal survival. PI3K and Akt are downstream from survival factors bound to membrane tyrosine kinase receptors on their perspective neuronal cell types [53]. These survival factors activate recruitment of PI3K and generate PIP₂ and PIP₃ at the cytoplasmic membrane, which leads to activation of several serine/threonine kinases, such as Akt. Akt is then recruited to the inner surface of the plasma membrane and is phosphorylated [53]. In hypoxic conditions, decreased Akt is a mechanism by which neuronal death occurs [52]. Akt reduces neuronal cell apoptosis through inhibiting caspase activation by cytochrome c [52]. Dexamethasone exerts neuroprotection through activation of phosphorylated Akt [52]. Interestingly, hypothermia treatment also shows neuroprotection by increasing Akt exerts activation [52], showing a common mechanism mediated by glucocorticoids (Figure 2).

However, it appears that dexamethasone does not decrease reactive oxygen species (ROS) production or affect antioxidant enzymes (glutathione peroxidase, and CuZn-, Mn-superoxide dismutase), suggesting that the neuroprotective effect of glucocorticoids focuses more on attenuating downstream mechanisms that cause damage to neuronal cells rather than on decreasing ROS [41,48].

Currently, only one study has looked at the role of MRs in adult cerebral ischemia and has suggested that suppression of MR-mediated mechanisms provides beneficial effects in an adult ischemic model [54]. However, in normoxic conditions, stimulation of microglial cells via corticosterone did not elicit cytotoxic effects or halt proliferation with MR stimulation [55]. MR activation alters transcription of L-type calcium channels and NMDA receptor activity, which leads to reduced calcium flux [56]. Moreover, MR signaling upregulates Bcl-2 and Bcl-xL, which stabilize the mitochondrial membrane and inhibit apoptosis [57]. Further studies are needed to explore the effect of MRs in the regulation of neonatal HI-injury.

Neurotoxic mechanism of glucocorticoids: The duration of glucocorticoid dosing is an important factor in determining neurotoxic or neuroprotective outcomes. When administered chronically (3-day dose), glucocorticoids act by different mechanisms than with a single-dose model. This difference in action merits discussion because it is key to understanding cellular adaptations to glucocorticoid administration under different circumstances in HI injury. Presently, the mechanisms underlying this difference of action remain largely elusive. A study that involved chronic and tapered dosing of glucocorticoid exposure before HI injury resulted in exacerbated neuronal cell death and white-matter injury [58]. This same study also showed evidence of immature oligodendrocyte apoptosis through caspase-3 activation after chronic dexamethasone treatment [58].

Extracellular glutamate accumulation in HI injury induces cytotoxicity and neuronal damage, which contributes to the deleterious effects seen in neonatal brain injury [59]. Glutamate-aspartate transporter (GLAST) and glutamate transporter-1 (GLT-1) are key

transporters in glutamate uptake and are chiefly found in glial cells, such as astrocytes. In a study with a 3-day pretreatment of dexamethasone before neonatal HI injury, glutamate uptake was decreased via reduced GLT-1 and GLAST [60]. Interestingly, NMDA receptors, which are activated by excitatory amino acids such as glutamate, were not altered by neonatal administration of dexamethasone [60]. It appears that glucocorticoid toxicity is mediated by increasing glutamate without upregulating NMDA and AMPA receptors, which are both sensitive to glutamate. Increased excitatory amino acids cause downstream influx of calcium and sodium into the cell, leading to apoptotic cellular death [61].

Furthermore, in neonatal HI, glucocorticoids might enhance apoptotic cell death via cyclin-dependent kinase 5 (CDK5) overexpression. CDK5 is a serine/threonine kinase that aids in cell cycle regulation. Present evidence suggests that Cdk5 is a prodeath signal in adult stroke models [62]. In neonatal hypoxia, p35 is cleaved by calpains to generate p25, which activates CDK5 [62]. When the p25/CDK5 complex is overactivated, phosphorylation of tau proteins and the GR causes heightened apoptosis. Inhibition of p25/CDK5 in HI injury halts the apoptotic cascade through reduced caspase-3 activity [62].

Innate immunity response

Microglia: Microglial cells, the resident myeloid cells in the central nervous system (CNS), are a type of glial cell derived from erythromyeloid progenitors that migrate to the developing brain starting on embryonic day 8.5 [63]. During their development, resting microglia are highly motile and ramified, with small cell bodies and long branched processes that act as surveillance in the developing brain microenvironment [63]. In addition, they have an active role in synaptic pruning, which is critical for neuronal maturation, neurogenesis, and homeostasis through clearance of apoptotic debris [63]. Microglial cells produce a robust inflammatory response to pathological conditions, such as neurodegeneration, autoimmune disorders, traumatic brain injury, and perinatal HIE [64].

During HI insult, damaged neuronal cells activate microglial cells, which morphologically transform to an 'activated' state, proliferate, and release proinflammatory mediators, such as TNF- α and IL-1 β , in as little as 2 h after initial HI insult, with peak microglial proliferation at 1 week [64,65]. These activated microglial cells, characterized as the M1 phenotype, release proinflammatory cytokine mediators (TNF- α , IL-1 β , IL-6, and IL-18) that cause secondary neuronal damage and axonal injury, known as white-matter damage, recruitment of lymphocytes, and exacerbation of neuronal damage [9,12,66]. However, microglial cells also have an equally pivotal role in the resolution of inflammation, with their switch to an anti-inflammatory phenotype [12]. After inflammatory resolution, microglial cells resolve inflammation, clean debris, and aid in tissue repair [67].

Given that microglial cell activation requires hours to days to fully develop, glucocorticoids could provide a neuroprotective response through downregulation of microglial-mediated inflammation (Figure 3). In the adult brain, corticosteroids are established as inhibitors of microglial activation in many neuropathologies, including traumatic brain injury and spinal cord injury [68,69]. Previous studies in the neonatal rat indicated that glucocorticoids reduced amoeboid microglial cells in the corpus callosum [70]. Surprisingly, only one study has addressed the potential use of corticosteroids in a hypoxic model. In the neonatal brain,

hypoxia-induced microglial activation was ameliorated with cortisone treatment [71]. However, the glucocorticoid was administered before ischemia in the study, and the therapeutic implications of post-ischemic administration of glucocorticoids should be explored further. Given that this area is understudied in the neonate, studies in the adult model provide supporting evidence in the role of GRs and MRs in modulating microglial function in brain HI injury.

Microglial cells in the adult brain highly express both GR and MR, with GRs being the most abundant steroid hormone receptor [51]. Furthermore, in the presence of an inflammatory challenge, such as lipopolysaccharide (LPS), microglial cells downregulate both GRs and MRs [51]. This appears to be an adaptive response in microglial cells to suppress anti-inflammatory signals in an effort to reach a full inflammatory state. Without steroid control, microglia cells would clear necrotic debris that could exacerbate injury. By contrast, highly reactive microglial cells, without a control system in place, could cause proinflammatory activation that might cause more harm than good. Interestingly, in an *in vitro* model, corticosterone significantly reduced both TNF- α and IL-6 secretion from microglia [51], giving additional evidence of microglial inactivation. The antiinflammatory response of microglial cells is glucocorticoid receptor mediated through the repression of key signaling pathways via dephosphorylation of PI3K, Akt, I κ B, and NF- κ B [73, 74], thereby protecting accumulation of detrimental cytokines and substances that lead to neuronal death and injury.

In an inflammatory state, the GRs in adult microglial cells are required for a controlled inflammatory response. Absence of GRs exacerbates LPS-induced inflammation and leads to neuronal degeneration, as well as stunting the proliferation, activation, and motility of reactive microglial cells [75]. Moreover, in resting microglia, there is a minimum level of signaling that is needed for cell survival and proliferation of both GRs and MRs [55]. Glucocorticoids enhance microglial activation and proinflammatory cytokine production, yet this study was established under chronic stress models [76] and did not provide mechanistic evidence of glucocorticoid action in acute inflammation and hypoxia. One study of LPS-induced inflammation reported that microglial GRs regulate Toll-like receptor 4 (TLR4) expression and upstream mediators, indicating that GRs have a critical role in reducing the deleterious effects of acute inflammation in infectious circumstances [75]. Current efforts mainly aim to develop a therapy that ameliorates microglial activity, rather than to understand the mechanism behind glucocorticoid-induced microglial suppression. Thus, mechanistic studies are needed in hopes of understanding how glucocorticoids halt microglial activity in neonatal HI injury. Furthermore, studies separating microglial action in adult ischemia versus neonatal ischemia are needed to understand the crucial role of microglia in initial stimulation of the immune system to subsequent healing after injury.

Astrocytes: Astrocytes are the most abundant cells in the CNS and have a major supportive role in neuronal health. Astrocytes, or astroglia, serve to metabolically support neuronal cells, maintain neurotransmitter homeostasis, support neurogenesis, regulate properties of the BBB, and help orchestrate neural plasticity [77].

In pathogenic states, astrocytes undergo a morphological transformation to reactive astrocytes, characterized by hypertrophy of the cell body and upregulated expression of glial

fibrillary acidic protein (GFAP) [77,78]. Reactive astrogliosis is detected from 4 to 7 days after ischemic insult [79]. Similarly, upregulation of GRs and MRs in reactive glial cells is detected 4 days after ischemic insult in the hippocampal CA1 region, with more prominent MR immunoreactivity [72]. In neuronal injury, astrocytes are thought to be responsible for glial scar formation in the damaged hemisphere [77]. They have a supportive role in repair through secretion of neurotrophic factors, such as brain-derived neurotrophic factor (BDNF), GDNF, nerve growth factor (NGF), basic fibroblast growth factor (bFGF), and neurotrophins 3,4, and 5 [54]. By contrast, astrocytes also mediate the innate immune response and aggravate nerve injury through the release of proinflammatory cytokines, such as TNF- α , IL-1 β , and IL-6 [12]. Moreover, immature neuronal astrocytes are sensitive to ischemic injury and are incapable of preventing excitotoxic damage through the regulation of glutamate homeostasis [13].

GRs are also major players in astrocytic regulation during ischemic brain damage (Figure 3). In HI injury, astrocytes become activated with subsequent reactive gliosis and upregulation of glial fibrillary acidic protein (GFAP) [77]. In normoxic conditions, dexamethasone attenuates GFAP expression and astrocytic proliferation [80], whereas, in hypoxic conditions, GFAP expression is increased [81].

Astrocytes cause a proinflammatory response to neonatal HI that results in the upregulation of intercellular adhesion molecule-1 (ICAM-1), IL-1 β , TNF α , IL-8, and monocyte chemoattractant protein-1 (MCP-1) in human endothelial cells [80]. In microvascular endothelial cells, astrocytic release of IL-1 β induces upregulation of ICAM-1, vascular cell adhesion molecule-1, and E-selectin. Upregulation of adhesion molecules allows for subsequent rolling and leukocyte transmigration into the damaged tissue from the cerebrovasculature [12]. Dexamethasone inhibits IL-1 β production by astrocytic neuronal cells, a key component in the activation of adjacent cells and proinflammatory stimulation [80]. This inhibits paracrine activation of the human brain endothelium, providing protection at the BBB.

The BBB can be compromised through an endothelin signaling mechanism [83]. In transient ischemic injury, astrocytic cells upregulate endothelin receptors and increase endothelin-1 (ET-1) [83]. ET-1 stimulates vasoconstriction and reduces cerebral blood flow. It increases the production of the matrix metalloproteinases (MMPs) 3, 9, and VEGF, which increases permeability of the BBB. In addition, it acts as an inflammatory mediator through release of the chemokines CCL2, CXCL1, and CX3CL1, and the cytokines IL-6 and TNF α [83,84]. In cultured astrocytes, glucocorticoids inhibit astrocyte activation through a reduction in ET_A and ET_B receptors, resulting in decreased ET-induced production of MMP3 and MMP9 [78]. Reduction in MMP3 and MMP9 helps protect the BBB, which decreases brain edema and reduces infiltration by inflammatory cells. Research has shown that MMP3 and MMP9 are inhibited by an ET_B antagonist, not an ET_A antagonist [83]. These results are consistent with a neonatal HI model in which ET_A inhibition failed to improve brain injury [85]. It is likely that ET_B antagonism will have beneficial effects in neonatal HI injury, although this has not yet been studied.

Neurotrophic signaling systems provide one avenue in which glucocorticoids are neuroprotective in cerebral ischemia. Astrocytic cells are most implicated in neurotrophin production and signaling. Neurotrophin receptor signaling, mediated by the Trk receptor, is diverse and thought to promote neuronal survival, growth, and differentiation [86]. Trk receptors bind the ligands NGF, BDNF, and other neurotrophins [86]. After an ischemic insult, upregulation of NGF and BDNF increases with a decline in neurotrophin-3 (NT-3) expression [81]. With dexamethasone treatment, NGF expression was transiently maximized 6 h after injury. In addition, dexamethasone-induction resisted a change in expression of NT-3 and BDNF up to 3 days after ischemic event. In normoxic conditions, dexamethasone activates phosphorylation of the TrkB receptor through a genomic mechanism without modifying the neurotrophins NGF, BDNF, and NT-3 [86], although a study showed upregulation of NGF in hippocampal neuronal cells and downregulation of NGF in astrocytes [87], resulting in a global picture of no change in NGF levels. Given that neurotrophins are potent prosurvival proteins, these findings suggest that glucocorticoids promote neuroprotection of astrocytic and neuronal cell populations through a neurotrophin-dependent mechanism of an increase in NGF, and through a neurotrophin-independent mechanism of genomic-mediated activation of the Trk receptor.

As discussed above, glutamate excitotoxicity accumulates extracellularly in HI injury, promoting cellular apoptosis and death. To avoid excitotoxic damage, GLAST and GLT-1 rapidly uptake glutamate and convert it to glutamine. In astrocytes, activation of GRs by both dexamethasone and corticosterone upregulates GLT-1 transcription, providing a potential role in glucocorticoid-mediated neuroprotection [88].

Interestingly, in ischemic injury, MR immunoreactivity was more prominent in astrocytes [72], possibly associated with neuronal survival and stability. Unfortunately, MRs have not been well studied in neonatal HI models and, therefore, the significance of astrocytic upregulation of the MR is merely speculative at this stage but should be studied further. In adult cerebral ischemia, MR antagonism is shown to have a beneficial effect, mainly mediated through astrocytic-mediated reduction in superoxide production, and increases in bFGF and VEGF [54]. Similarly, astrocytic production of VEGF is inhibited by glucocorticoids [89]. Upregulation of bFGF and VEGF increased angiogenesis and recruitment of additional astrocytes, which aids in support of damaged tissue and a reduction in ischemic volume. It is possible that increased neovascularization helps recruitment of neuroblasts into the damaged striatum; bFGF is a prosurvival signal that protects neuronal cells from damage [54]. However, it is unknown how MRs and GRs will change with a circumstance shift, such as neonatal HI injury. Studies indicate that MR agonism is neuroprotective in the neonatal brain (Concepcion and Zhang, unpublished observations). Although the mechanism remains unknown, it is likely that neuroprotection is mediated by neuronal astrocytes.

Neutrophils: Leukocyte recruitment begins with upregulation of cell adhesion molecules in the injured endothelium. In the vascular bed, selectins mediate ‘rolling’ of leukocytes along the endothelial wall, with integrins mediating ‘adhesion’. Microvascular selectins can be transcriptionally upregulated by the inflammatory mediators IL-1 β and TNF α [90, 91]. In an inflammatory state, NF- κ B is transcriptionally upregulated in endothelial cells, which

induces cytokine expression, ICAM-1, and E-selectin [90,91]. Glucocorticoids are thought to inhibit adhesion molecules and cytokines (IL-6, IL-8, endothelin-1, and VEGF) expression via transcriptional downregulation of NF- κ B [91].

Neutrophils are the most abundant white blood cells found in circulation and protect against infection through increased phagocytosis, oxidative bursts, and later resolution of inflammation [92]. In ischemic injury, neutrophil and macrophage activation releases free radicals, which cause free radical oxidative damage and render the neonatal brain vulnerable through oligodendrocyte and neuronal injury [93]. Neutrophils first appear in the cerebral vasculature 4 h after HI injury in the neonatal rat brain. In addition, neuroprotection only occurs with complete neutrophil deprivation before HI brain injury [14]. In a model of renal ischemia, glucocorticoids downregulate neutrophil infiltration via downregulation of ICAM-1 [90], indicating a mechanism of glucocorticoid protection in decreasing neutrophilic extravasation. In normoxic conditions, glucocorticoids inhibit neutrophil cell adhesion to endothelial cells and reduce myeloperoxidase (MPO) and superoxide anions [94], providing insight into glucocorticoid protection of neuronal cell populations. Conditional to microenvironmental changes, dexamethasone does not prolong neutrophil survival in hypoxic-inflammatory conditions, yet without inflammation dexamethasone promotes neutrophil survival [95], which could be problematic at the resolution phase of injury.

Macrophages/monocytes: Macrophages are blood-borne cells that are classically described as activated macrophages (M1), accompanied by proinflammatory markers, and alternatively activated macrophages (M2), accompanied by anti-inflammatory and homeostatic functions [96]. In hypoxic injury, circulating, activated macrophages infiltrate the ischemic parenchyma, and release proinflammatory cytokines and ROS, although they also have a role in orchestration of tissue repair [12]. Glucocorticoids prevent leukocyte-endothelial adherence, suggesting that it has a role in neonatal HI pathology [97]. Interestingly, glucocorticoids also prevented the respiratory burst and release of MCP-1 following hypoxia [97]. Downregulation of MCP-1, a selective monocyte attractant, is a promising target in the protection of ischemic injury [98]. In neonatal HI injury in the rat, MCP-1 is detected within 1 h after injury. By contrast, in human neonates, MCP-1 is increased within 9 h of HI injury and is associated with severely abnormal neurodevelopmental outcomes [7,98]. Glucocorticoids attenuate macrophage accumulation in the ischemic brain via reduced MCP-1 expression, yet have no influence over neuronal damage [99]. Monocytic attenuation might be a combination of a plethora of mechanisms caused by glucocorticoids, including decreases in peripheral monocyte population and leukocytic infiltration, attenuation of chief monocytic attractant MCP-1, and prevention of the respiratory burst [97,99].

Adaptive immunity response—Lymphocytic cells (CD4⁺ and CD8⁺ T cells) infiltrate the neonatal brain as soon as 12 h after HI injury and can persist in the damaged hemisphere for up to 42 days in the neonatal rat [100]. Professional antigen cells, known as dendritic cells, are also detected in neonatal HI brain, characterized by upregulation of CD11b⁺ and CD11c⁺ with co-stimulatory upregulation of CD86 and MHC-II [100]. At 1 week post-ischemic injury, naïve CD45rb⁺T lymphocytes were found in the damaged brain hemisphere

with CD45rb⁺ T lymphocytes persisting at 3 months after injury [100]. These T regulatory cells have implications in anti-inflammatory mediation and homeostasis. The role of glucocorticoids in these populations has not yet been explored. Recent evidence confirmed that GR expression on CD8⁺ T cells is critical to control overactivation of inflammatory mediators [101]. Evidence indicates that dexamethasone downregulates HIF-1 α induction in human hypoxic CD4⁺ T cells [102]. The role of glucocorticoids in the long-term response has not yet been well studied.

Chemokines and local mediators—Cytokines In HIE, the neonatal brain expresses a unique phenotype resulting from the immaturity of the neonatal immune system [67]. In HI injury, initial injury and apoptosis of neuronal cells release endogenous molecules, damage-associated molecular pattern molecules (DAMPs), that trigger pattern recognition receptors (PRRs), such as TLRs, of the innate immune system to induce an initial inflammatory response [67]. TLR-3, activated by double-stranded (ds)DNA, exacerbates neonatal HI injury after initial neuronal damage through initiation of NF- κ B signaling, increase in proinflammatory genes, and reduction of the anti-inflammatory M2-microglial phenotype [103]. Within 24 h, IL-1 β , IL-6, and TNF- α are increased in the cerebrospinal fluid (CSF) in neonates with HIE [104,105]. Of importance, cell death and apoptosis positively correlate with increases in TNF- α and IL-1 β [106].

In HI injury, TNF- α is secreted by microglia cells, astrocytes, and neuronal cells. TNF- α is readily detectable immediately after HI injury [107]. In addition, astrocytic release of TNF- α prolongs the inflammatory response [108]. TNF- α mediates the upregulation of the adhesion molecules ICAM-1 and VCAM-1 to aid leukocyte recruitment [109]. It also increases apoptosis via caspase-3 activation in microvascular endothelial cells, neurons, and oligodendroglial precursor cells. In addition, it contributes to BBB disruption via TNF receptor 1 (TNFR1) because both BBB breakdown and cleaved caspase-3 are ameliorated by TNFR1 knockout in the mouse model [110]. Through inhibition of NF- κ B, glucocorticoids inhibit TNF- α [111]. Interestingly, in activated microglial cells, high doses of corticosterone significantly reduce TNF- α secretion, whereas it is ineffective at lower doses [51]. In an adult ischemic model, glucocorticoids provide neuroprotection with reduced TNF- α in the ipsilateral hemisphere, with subsequent attenuation of the inflammatory reaction and apoptosis [50,112].

In the neonatal rat, IL-1 β increases 3–4 h after HI damage [107,113,114]. IL-1 β levels in the CSF or umbilical cord plasma of a newborn with HIE correlate with disease severity and abnormal neurological outcomes [104,115]. IL-1 β is implicated as the chief cytokine that induces the cascade leading to neuronal injury at various stages of inflammation in HI damage [116]. Recent evidence showed that IL-1 receptor antagonism is neuroprotective and has potential for the treatment of HI injury [114,117]. Blockade of the IL-1 receptor (IL-1R) after HI damage significantly reduced cell death and caspase-3 activation by inhibiting NF- κ B transcriptional activity [114]. The anti-inflammatory effects of glucocorticoids are thought to be mediated via genomic mechanisms. Through inhibition of NF- κ B, glucocorticoids inhibit both cytokine IL-1 β and IL-1R in inflammatory conditions [80,111]. Interestingly, it has been shown that IL-1 production mediates endogenous glucocorticoid induction. In IL-1 β -knockout mice, glucocorticoid induction was stunted 8 h after initiation

of inflammation, suggesting that IL-1 β suppresses the inflammatory reaction through control of glucocorticoids during the later stages of injury [118]. With this evidence, studies using a neonatal HI model are needed to understand glucocorticoid control in the immature neuronal immune response and if similar mechanisms take place in the neonatal brain.

In patients with HIE, high levels of IL-6 detected in the CSF significantly correlated with subsequent brain injury and adverse neurological outcomes at 2 years of age [105,119]. Patients with extremely elevated IL-6 serum levels experienced more severe injury and had higher mortality rates [7]. High-dose corticosterone significantly reduced IL-6 [51]. In the adult ischemic model, glucocorticoids reduce IL-6 mRNA and protein levels after ischemic injury [112]. Interestingly, previous studies involving IL-6 were merely correlative and had yet to test the effect of IL-6 activation or inhibition in the pathogenesis of HI injury. One recent study in the ovine fetus validated IL-6 neutralization as neuroprotective through attenuation of ischemia-induced BBB permeability [120]. It was proposed that the mechanisms were involved the modulation of the tight junction protein, plasmalemma vesicle protein [120]. Thus, IL-6 provides another promising avenue of neuroprotective therapies for neonatal patients, although this needs to be studied further.

In addition, the anti-inflammatory cytokine IL-10 has a crucial role in tipping the balance towards a neuroprotective outcome in HI injury. IL-10 overexpression in mice showed neuroprotection in cerebral ischemia. These mice expressed significantly reduced active caspase-3 and reduced HIE-induced neuronal apoptosis [121]. IL-10 inhibits IL-1, IL-6, and TNF- α production from glial and astrocyte cells [122,123]. Furthermore, IL-10 is involved in the regulation of adult neurogenesis [124]. Glucocorticoid administration significantly upregulates IL-10 production via NF- κ B transcription [111]. Interestingly, persistently elevated IL-10 cytokine levels correlate with severe neurological outcome, although this could be a correlation with irreversible injury [7]. In summary, IL-10 is a promising candidate to increase neuroprotection in patients HIE, although more studies are needed to explore the effects of IL-10 in neonatal health, specifically its effects on neurogenesis and immune dysregulation.

Chemokines: Chemokines, chemotactic cytokines, are primarily responsible for mediating recruitment of leukocytes during an inflammatory insult. Following neonatal HI injury, the immature immune system activates an inflammatory immune response by deploying both α -chemokines and β -chemokines from the endothelium and neural tissue to recruit key immune cells [12,100]. In rat neonatal HI injury, α -chemokines promote recruitment of neutrophils within the first 12 h of HI insult, whereas β -chemokines are thought to recruit microglia/macrophages, CD4⁺ and CD8⁺ lymphocytes that persist in chronic inflammation [12,100]. Given that chemokines have a major role in inflammatory cell recruitment, it would be beneficial to explore the effect of glucocorticoid control on key players of chemokines in HI injury.

Stromal cell-derived factor-1 α (SDF-1 α), also known as CXCL12, is produced by neuronal cells and astrocytes during central inflammation in the perivascular area of HI injury in the neonatal mouse [125]. Signaling of SDF-1 α is mediated by the receptor CXCR4, a G_i-protein-coupled receptor that regulates ERK signaling and downstream Ca²⁺ mobilization in

cortical type I astrocytes [126]. SDF-1 α is an α -chemokine that is a candidate inflammatory marker in the recruitment of immune cells and exacerbation of ischemic injury in adult stroke [127]. Tangentially, SDF-1 α increases injury repair through recruitment of progenitor cells for neurogenesis, neuroblast migration, and ischemic neovascularization during the recovery phase of HI injury [128]. In neonatal HI injury, dexamethasone downregulates chemokine receptor CXCR4, exerting a neuroprotective effect with diminished astrocytosis in areas of HI damage in the rat [129]. More recently, in the adult mouse stroke model, CXCR4 antagonism significantly reduced the inflammatory response by inhibiting leukocyte migration and proinflammatory cytokine expression [130]. Inhibition of SDF-1 α /CXCR4 by dexamethasone results in an anti-inflammatory response through the reduction of reactive astrocytes and CXCR4 receptor density, thereby indicating a neuroprotective role in the neonatal brain exposed to HI. However, because SDF-1 α /CXCR4 has a role in neurogenesis during later stages of wound repair, more studies are needed to explore whether glucocorticoids cause a transient reduction in CXCR4 that would still allow for the migration of neuronal and endothelial progenitor cells for brain recovery.

A chemokine that is established in neonatal HI injury as a main chemotactic factor and might be a promising target of glucocorticoids, is chemokine ligand 2 (CCL2), also known as MCP-1. CCL2, a β -chemokine, binds specifically to its receptor chemokine receptor type 2 (CCR2), a G-protein-coupled receptor that is expressed on endothelial cells and neural cells [131]. In neonatal HI injury, CCL2 is abundantly expressed in reactive astrocytes and microglia adjacent to areas of injury [132]. CCL2/CCR2 signaling recruits monocytes, T cells, and natural killer cells to areas of inflammation [133]. Importantly, when evaluating clinical outcomes of neonates with HI injury, high CCL2 levels significantly predict poor clinical outcome [7]. In the adult mouse, CCR2^{-/-} protected against ischemic injury through a reduction in monocyte and neutrophil infiltration [134]. Additionally, in the adult ischemic mouse model, CCL2^{-/-} mice showed reduced injury at the site of infarction [135]. Of interest, in a model of alveolar hypoxia, glucocorticoids blocked the hypoxia-induced release of CCL2 and leukocyte extravasation [97]. It is possible that glucocorticoids suppress CCL2 production in the brain through a similar mechanism, although studies are needed to further explore the mechanism through which the GR regulates CCL2.

In addition, CXCL8 (IL-8), is an α -chemokine that is upregulated in the serum and CSF in neonates with HIE [7]. Elevated IL-8 during the early stage of HIE is associated with abnormal neurological outcomes and correlates with the severity of injury [136]. Hypoxic upregulation of IL-8 via a NF- κ B mechanism modulates leukocyte chemotaxis and neutrophil transmigration [137]. There is evidence that suggesting that HIF-1 α induces corticosteroid-insensitive inflammation that renders CXCL8 unbeatable by synthetic glucocorticoids [138], although this has not yet been studied in neonatal HI injury. In renal epithelial cells, hypoxia upregulates the GR and sensitizes glucocorticoid-treated cells, causing inhibition of IL-8 production [32].

Epigenetic control of GR and MR in HI neonatal brain injury—Epigenetic modifications are inheritable patterns that control gene expression without changing the genetic code. Epigenetic modifications that occur in hypoxic injury include DNA methylation and histone modifications, as well as short or long noncoding RNAs [27].

Methylation in the regulatory regions of DNA is associated with suppression of gene transcription [27]. Interestingly, endogenous glucocorticoids induce epigenetic changes that leave their footprint on the developing fetus [139]. Evidence indicates that the endogenous glucocorticoid surge during late gestation modifies methylation patterns in the hippocampus, leading to reprogrammed DNA binding, methylation, and transcription of GRs [140]. Importantly, there is an epigenetic ‘switch’ that occurs during late gestation that orchestrates the GR promoter region [27]. Alternatively, synthetic glucocorticoid exposure alters the binding of the GRE and may alter GR DNA binding in the fetal hippocampus, although it is unclear whether these changes persist throughout fetal life [140]. Maternal hypoxic injury causes DNA methylation with binding of methyl-CpG-binding proteins (MBDs) to exon promoter regions of the GR gene [141]. As a result, dexamethasone confers reduced neuroprotection through epigenetic downregulation of GRs [141].

MiRNAs are another epigenetic mechanism to regulate translation of target proteins. They are typically approximately 20-base pairs long and target mRNA at the 3′ untranslated region (UTR), resulting in degradation of target mRNAs or repression of translation [142]. In the adult rat, miR-210 increases 24 h after transient ischemia injury [143]. Studies showed that miR-210 in HI injury had a neuroprotective role by suppressing neural apoptosis, inhibiting caspase activity, and reducing brain edema [144,145]. However, recent studies found that miR-210 targets GR expression at the 3′-UTR in the hippocampal region, thereby priming the neonatal brain to injury [146]. Inhibition of miR-210 produced a neuroprotective effect and improved long-term neurological functions [146]. Furthermore, miR-210 inhibition reduced the proinflammatory cytokines TNF- α , IL-1 β , and IL-6, and chemokines CCL2 and CCL3, which further suggests a role in controlling disease severity [12]. Timing of miR-210 expression is of interest because a study in normoxic conditions suggested that miR-210 expression promotes angiogenesis and neurogenesis [148].

Few other miRNAs have been connected to glucocorticoids during HI injury, yet there are potential candidates worth mentioning. Neuronal exposure to glucocorticoids results in BDNF suppression via a decrease in miR-132 expression, possibly with a role in weakened synaptic function [149]. Interestingly, hypoxia increases expression of miR-132 and increases Schwann cell migration in peripheral nerve injury [150]. In hypoxic cardiomyocytes, miR-132 overexpression prevented Ca²⁺ overload with a subsequent decrease in apoptotic protein expression [151]. Further studies are needed to explore the effects of glucocorticoids on miR-132 in hypoxic conditions and whether they are a part of the neuroprotective strategy. Other miRNAs, such as miR-449a, -182, -124, and -186, are upregulated in a hypoxic environment and are connected to glucocorticoid regulation and control of neural cells, yet their role in neonatal brain injury remains largely elusive [152–156].

Therapeutic implications of corticosteroids

A question that remains is whether glucocorticoids are neuroprotective or neurotoxic in neonatal HI brain injury. This controversial is primarily the result of the differences seen in dosing, timing, method of administration, and severity of the model used (Table 1). In some animal models of neonatal brain HI injury, pretreatment with glucocorticoids, such as

dexamethasone, has shown a neuroprotective effect [41,48,52,129,157,158]. Although these findings are promising, pretreatment of glucocorticoids does not provide a realistic means of translating these findings because pre-injury assessment of HI is difficult [159]. By contrast, evidence shows that post-HI injury treatment with dexamethasone can be both neurotoxic and neuroprotective [15,16,159]. Interestingly, glucocorticoid treatment was neurotoxic in cases where dexamethasone treatment was provided subcutaneously (0.1 mg/kg or 0.5 mg/kg), whereas it was neuroprotective when it was administered directly into the brain by i.c.v. injection or through intranasal administration [15,159]. It is likely that systemic effects and resultant adverse effects of glucocorticoids might modify the neuronal apoptotic or inflammatory cascade differently through HPA axis dysregulation, compared with the improved success of i.c.v. and intranasal drug delivery.

Concluding remarks

Perinatal HI brain injury is a devastating disease that causes severe long-term neurological problems. The molecular mechanisms and pathway of brain injury in infants with HIE remain largely elusive. Although therapeutic hypothermia is the current standard of care for term newborns with moderate to severe HIE, nearly half of affected infants treated with hypothermia still die or suffer significant neurological disability. An area of high priority for study is to develop sufficient experimental knowledge to warrant assessment of adjuvant therapies to hypothermia, to improve outcomes of HI brain injury in the neonate. Glucocorticoids provide a potential adjuvant therapy to hypothermia, through modulation of apoptosis and inflammation in response to injury in the neonatal brain. The ability of glucocorticoids to act as a suppressor to neuroinflammation is dependent on timing, dosing, and duration of exposure after initial injury. Although current studies provide limited information about the role of GRs and MRs in HI brain injury, promising findings suggest that the adjuvant therapy of single-dose glucocorticoid administration alongside hypothermia might have synergistic effects in terms of minimizing ongoing brain injury, improving the outcome of patients with HIE and the long-term optimization of infant neuroplasticity.

Teaser: Glucocorticoids modulate inflammation and apoptosis in the neonatal brain, providing potential therapeutic strategies that could be beneficial for the treatment of infants with HI brain injury.

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AUTHOR BIOGRAPHY

Katherine R. Concepcion: Katherine R. Concepcion received her BA degree in Molecular and Cell Biology from University of California, Berkeley in 2013 and is currently pursuing her MD/PhD at Loma Linda University. Her graduate studies are supported through the Loma Linda University for Maximizing Student Development Program through the National Institutes of Health. Her research focuses on glucocorticoid mechanisms in hypoxic-ischemic injury in the neonatal brain.

Lubo Zhang: Dr. Zhang is professor of Pharmacology and Physiology and Director of Center for Perinatal Biology at Loma Linda University School of Medicine. He was the President of the Western Pharmacology Society in 2008. He has been members in the various study sections of grant review for US National Institutes of Health for more than 20 years. Dr. Zhang is the author/coauthor of over 600 scientific articles, book chapters and abstracts. His research interests focus on the molecular and epigenetic mechanisms in developmental programming of health and disease.

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Highlights

- Neonatal HIE is the major cause of neonatal mortality and neurologic morbidity.
- Glucocorticoids modulate inflammation and apoptosis in the neonatal brain.
- Glucocorticoids provide a potential adjuvant therapy to hypothermia in the treatment of HIE.

Figure 1. Representation of glucocorticoid receptors (GRs) and mineralocorticoid receptors (MRs) during brain development. In the embryonic rat, GRs are diffusely present in the brain at embryonic (E) day 12.5, including the embryonic hippocampus, as shown. In the mouse, GRs are first detected in the hippocampus at postnatal (P) day 1, whereas MRs are first detected at E15.5 in the CA 1-3 region. Analogous to the third trimester in humans P1 in both rodent models (rat and mouse) shows substantial MR expression in the hippocampus, with GR expression present in the CA1-2 region. The time point most frequently used in the

hypoxia-ischemia rodent model is P7, because the rat neonatal brain is most similar in morphology to the human brain at term (38–40 weeks). It is at this time point that the rat model closely resembles MR and GR expression in humans, with the mouse showing decreased GR expression in the CA3 region. Abbreviations: CA, cornu ammonis; DG, dentate gyrus.

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Figure 2.

Schematic representation of glucocorticoid control of key apoptotic proteins in cellular death in hypoxic-ischemic (HI) injury. In HI injury glucocorticoids are neuroprotective in the neonatal brain by targeting key apoptotic pathways. First, glucocorticoids preserve the glycolytic flux, maintain ATP demand, and resist intracellular calcium accumulation. Glucocorticoids also reduce intrinsic (mitochondrial-mediated) apoptosis by increasing phosphorylated Akt, thereby decreasing the release of cytochrome c. Additionally, mineralocorticoid agonists increase the prosurvival mitochondrial proteins Bcl-2/Bcl-xL. Glucocorticoids resist extrinsic (death receptor-mediated) apoptosis by decreasing the key death receptor ligand, TNF-α and downstream cleavage of caspase-3. Abbreviations: ETC, electron transport chain; GCs, glucocorticoids; MR, mineralocorticoid receptor; TNF, tumor necrosis factor; TNFR, tumor necrosis factor receptor.

Figure 3.

Schematic representation of glucocorticoid control of inflammatory mechanisms as a result of hypoxic-ischemic (HI) injury. In an inflammatory state, microglial cells release the proinflammatory mediators TNF- α and IL-1 β , resulting in secondary neuronal damage, white-matter damage, and recruitment of lymphocytes. Glucocorticoids downregulate activation of microglial cells and decrease the release of key proinflammatory cytokines that cause neuronal damage and later brain injury. Astrocytes, the most abundant cells in the central nervous system, are also targeted by glucocorticoids in an inflammatory reaction by protecting the brain through upregulation of neurotrophic factors that are critical for repair, downregulate proinflammatory cytokines, and prevent excitotoxic damage through the preservation of glutamate homeostasis. Lastly, glucocorticoids downregulate endothelin receptors, resulting in decreased matrix metalloproteinase (MMP) release and disruption of the blood-brain barrier (BBB). Lastly glucocorticoids block downstream upregulation of adhesion molecules and leukocyte recruitment by blocking astrocytic and microglial IL-1 β production. Abbreviations: ET, endothelin; GCs, glucocorticoids, GFAP glial fibrillary

acidic protein; GLT, glutamate transporter; ICAM, intracellular adhesion molecule; IL, interleukin; NGF, nerve growth factor; TNF, tumor necrosis factor.

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Table 1.Glucocorticoids as a therapeutic option for perinatal HI injury^a

Experimental insult and animal model	Intervention	Drug Administration	Time point of data collection	Outcome	Refs
Synthetic glucocorticoid (dexamethasone)					
HI, P7 rat; 8% O ₂ , 1.5 h	Dex given 5 h before insult	s.c. 0.5 mg/kg	48 h after HI	Neuroprotective with attenuation of chemokine receptor CXCR4 density and decrease in binding of SDF-1 α	[129]
HI, P7 rat; 8% O ₂ , 2 h	Dex given in tapering doses from P1 to P3	Three doses i.p.: 0.5 mg/kg on P1; 0.3 mg/kg on P2; 0.1 mg/kg on P3	7 d and 14 d after HI	Exacerbated white-matter injury through evidence of reduced myelin thickness, axon caliber, and function through astrocytic activation, and apoptosis in oligodendrocytes	[56]
		Three doses i.p.: 0.5 mg/kg on P1; 0.3 mg/kg on P2; 0.1 mg/kg on P3	24 h after HI	Exacerbates damage and reduces GLT-1	[60]
	Dex given 4 h before insult	One dose: i.p. 0.6 mg/kg	Long-term behavioral study (18 weeks)	Neuroprotective through prevention of histological brain damage and learning/memory impairment	[160]
	Dex given 24 h before insult	One dose: i.p. 0.1 mg/kg	During HI or 5 min after HI	Neuroprotective with Dex removing suppression of cerebral protein synthesis	[161]
			2 d after HI	Neuroprotective. Dex-only provides more protection than Dex with glucocorticoid antagonist	[40]
	Low- or high-dose Dex given for 4 consecutive days before insult	Four doses: low dose (i.p. 0.1 mg/kg/day); high dose (i.p. 0.5 mg/kg/day)	3 d, 7 d, 14 d, and 21 d after HI	Neuroprotective with high-dose Dex reducing mature neurons 7 days after injury, yet morphological differences disappear by 21 days after HI	[162]
HI, P7 rat; 8% O ₂ , 2-2.5 h	Dex given 2 h after insult	One dose, i.c.v., 0.1 μ g	48 h after HI	Neuroprotective	[15]
HI, P7 rat; 8% O ₂ , 2.3 or 2.6 h	Dex given 24 h and 4 h before insult	Two doses: i.p. 0.25 mg/kg/dose	24 h and 22 d after HI	Neuroprotective and augments VEGF mRNA and protein, reduces caspase-3 activity and cell death, and increases Akt phosphorylation	[1630]
HI, P7 rat; 8% O ₂ , 2.5 h	Dex given 1 h after insult	One dose: s.c. 0.1 mg/kg or s.c. 0.5 mg/kg	3 h and 24 h after HI	Exacerbated injury with elevated brain edema, reduced ACTH levels, and increased	[159]

Experimental insult and animal model	Intervention	Drug Administration	Time point of data collection	Outcome	Refs
				corticosterone blood plasma levels	
	Dex given 24 h before insult	One dose: i.p. 0.1 mg/kg	1 h, 24 h and 72 h after HI	Neuroprotective and antiapoptotic through evidence of attenuated <i>c-fos</i> , without alteration in Bcl-2 and ROS	[48]
HI, P7; 8% O ₂ , 2.6 h ²	Dex given 24 h and 4 h before insult	Two doses: i.p. 0.25 mg/kg/dose	24 h and 22 d after HI	Neuroprotective and prevents apoptosis by increased PI3K/Akt activity and attenuated cleaved caspase-3	[52]
HI, P7 rat; 8% O ₂ , 3 h	Dex given 22 h before insult	One dose: i.p. 0.1 mg/kg	During HI to 24 h after HI	Neuroprotective through preservation of cerebral energy metabolism through ketogenesis	[158]
	Dex given 24 h, 48 h, and immediately before insult	Three doses: i.p. 0.5 mg/kg/dose	2 h, 18 h, and 24 h after HI	Neuroprotective and antiapoptotic in CA1 hippocampal region with associated attenuation of <i>c-fos</i> and <i>c-jun</i>	[164]
	Chronic: Dex given 48 h, 24 h, and immediately before insult; one dose: Dex given either 24 h, 3 h, or immediately before hypoxia	Chronic (three doses): i.p. 0.5 mg/kg/day; one dose i.p. 0.1 mg/kg	7 days after HI	Neuroprotective: chronic, single-dose pretreatment, and single-dose post treatment prevented HI-associated brain damage	[157]
HI, P7/P14/P30 rat; P7 8% O ₂ , 3 h; P14 8% O ₂ , 1 h; P30 8% O ₂ , 0.5 h	Dex before insult, or 4 d, 48 h, or 3 h before HI	One dose i.p. 0.1 mg/kg	48-72 h after HI	Neuroprotective when Dex given 3-48 h before insult with protection in P7 and P14 rats; independent of blood glucose levels	[165]
HI, P10 rat; 8% O ₂ , 2 h	Dex given before insult	One dose; i.c.v., 2 µg/kg	48 h after HI	Neuroprotective through stimulated L-PGSD-dependent PGD ₂ biosynthesis via DP ₁ receptor activation and downstream pERK-44 activation	[16]
HI-LPS, P7 rat; 8% O ₂ , 1 h	Dex given 4 h before insult	One dose: i.p. 0.5 mg/kg	Long-term behavioral study	Neuroprotective in preventing short-term memory, long-term memory, and attention deficits with protection against adverse histological changes in cortex, striatum, and hippocampus	[166]
Hydrocortisone					
HI, P7 rat; 8% O ₂ , 2-2.5 h	Hydrocortisone given 2 h after insult	One dose: i.c.v. 10 µg; intranasal 300 µg	48 h after HI	Neuroprotective; high dose by intranasal	[15]

Experimental insult and animal model	Intervention	Drug Administration	Time point of data collection	Outcome	Refs
				administration elicited protection	
Cortisone					
HI, 7.7% O ₂ , 1.6 h	Cortisone given 24 h after birth	One dose; 5 mg/100 µl in nuncal region	3 days after HI	No change; decline in microglial cell numbers without change in GFAP with enhanced MHC class I expression and drastic reduction in astrocytic proliferation	[69]

^aAbbreviations: CXCR, CXC chemokine receptor; Dex, dexamethasone, E embryonic day; i.p., intraperitoneal; MHC, major histocompatibility complex; P, postnatal; PGD, prostaglandin; s.c., subcutaneous.