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Animal Models of Glucocorticoid-Induced Glaucoma

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Abstract

Glucocorticoid (GC) therapy is widely used to treat a variety of inflammatory diseases and conditions. While unmatched in their anti-inflammatory and immunosuppressive activities, GC therapy is often associated with the significant ocular side effect of GC-induced ocular hypertension (OHT) and iatrogenic open-angle glaucoma. Investigators have generated GC-induced OHT and glaucoma in at least 8 different species besides man. These models mimic many features of this condition in man and provide morphologic and molecular insights into the pathogenesis of GC-OHT. In addition, there are many clinical, morphological, and molecular similarities between GC-induced glaucoma and primary open-angle glaucoma (POAG), making animals models of GC-induced OHT and glaucoma attractive models in which to study specific aspects of POAG.

Keywords

glucocorticoid; ocular hypertension; glaucoma; intraocular pressure; steroid glaucoma

Glucocorticoid Therapy

The development and use of glucocorticoids (GCs) has been a major advance in the treatment of a wide variety of inflammatory and immune mediated diseases. In fact, GCs are unsurpassed in their potent anti-inflammatory and immunosuppressive actions because they intervene in these processes at multiple steps. GCs have been one of the most widely prescribed medications. Cortisol is the endogenous GC in man, whereas corticosterone is the endogenous GC in rodents, and both of these compounds have glucocorticoid and mineralocorticoid activities. A number of synthetic GCs have been designed to eliminate mineralocorticoid activity and to increase GC potency and half-life. GCs are administered via a spectrum of different routes, including: oral, intravenous, intra-articular, topical, inhalation, nasal, etc. GCs are used to treat a variety of ocular diseases, involving inflammation in almost all tissues of the eye such as: eyelids, conjunctiva, cornea, sclera,

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uvea, retina, and optic nerve. GCs are prescribed for these disorders via oral, topical ocular, subconjunctival/sub-Tenon's injections, intravitreal injections and implants, and periocular injection routes of administration.

Although GCs are clinically important and potent therapeutic agents, prolonged GC therapy can cause a number of serious side effects (Table 1), including the ocular side effects of posterior subcapsular cataracts and GC-induced ocular hypertension (OHT) and iatrogenic open-angle glaucoma. These ocular side effects can occur in some individuals regardless of the route of administration, although they are more prevalent in patients receiving intravitreal sustained GC delivery devices (Bollinger et al., 2011; Kiddee et al., 2013).

GC-induced Ocular Hypertension and Glaucoma in Man

GC-induced glaucoma (i.e. steroid glaucoma) is clinically very similar to primary open angle glaucoma (POAG), and diagnosis often relies on determination of whether the patient is currently undergoing GC therapy. GC therapy for weeks to months can painlessly elevate IOP in some individuals. If untreated, this OHT can lead to glaucomatous optic neuropathy and retinopathy without gonioscopic peculiarities, characteristic of POAG. GC-induced OHT is dependent on: duration of therapy, potency and physicochemical properties of the GC, route of administration, and individual susceptibility. OHT is generally reversible after discontinuation of GC therapy. However, vision loss due to this OHT is not reversible.

Not everyone receiving GC therapy develops GC-induced OHT. Approximately half of the individuals exposed to high intraocular levels of GCs, such as sustained intravitreal GC delivery devices, develop OHT (Bollinger et al., 2011; Kiddee et al., 2013). In early studies in the 1960s, individuals were given topical ocular dexamethasone or betamethasone (0.1% 3-4x/day) for 4-6 weeks to determine how many developed OHT ("steroid responder" rates). 4-6% of these individuals had significantly increased IOP, while approximately one third had had more modest pressure increases (Armaly, 1963a; Becker, 1965). In contrast, almost all POAG patients were considered to be steroid responders (Armaly, 1963b). The responder rate is much higher in patients receiving intravitreal sustained GC delivery implants, many of whom require glaucoma filtration surgery to treat GC-induced OHT so as to prevent iatrogenic glaucoma (Bollinger et al., 2011). It should be noted that steroid responsiveness has been reported to be heritable (Armaly, 1965; Becker, 1965), and steroid responders are at elevated risk for developing POAG (Kitazawa and Horie, 1981; Lewis et al., 1988). Interestingly, relatives of POAG patients have higher rates of steroid responsiveness (Paterson, 1965; Becker and Chevette, 1966; Davies, 1968; Bartlett et al., 1993). GC-OHT has been shown in perfusion cultured human anterior segments, an ex vivo model that mimics certain features of GC-OHT in man. The steroid responder rate in this isolated system was 30%, similar to that reported in clinical studies (Clark et al., 1995b).

Mechanisms of GC-Induced OHT

GC-induced OHT is due to increased aqueous humor outflow resistance that coincides with morphological changes within the TM. As TM cells express GC receptors, GCs appear to act directly on TM cells to influence cellular, biochemical and molecular changes that contribute to the development of TM outflow obstruction. Understanding the basis for GC-

induced changes in the TM would serve to clarify the pathogenesis of GC-induced OHT and suggest new approaches to treat GC-induced glaucoma.

GC-Induced Changes to TM Morphology and ECM

The mechanism of GC-induced outflow obstruction in the TM is not well understood, but appears to be associated with accumulation of extracellular matrix (ECM) material, particularly in the juxtacanalicular tissue (JCT) and along the inner wall endothelium of Schlemm's canal (SC) where the bulk of outflow resistance is presumably generated (Lütjen-Drecoll, 1973; Mäepea and Bill, 1992). In human TM from eyes with a diagnosis of GC-induced glaucoma, there is an accumulation of "fingerprintlike" material resembling coiled basement membrane material in the JCT with an abnormal accumulation of densely packed fine fibrils underneath the inner wall of SC (Rohen et al., 1973; Johnson et al., 1997), and a thinning of SC cells (Kayes and Becker, 1969). Similar fingerprintlike deposits are observed in the JCT of eyes with juvenile glaucoma (Furuyoshi et al., 1997). These ECM deposits in GC-induced glaucoma are distinct from the characteristic sheath-derived plaques that surround the JCT elastic fibers in POAG, with a greater accumulation of type IV collagen, heparin sulfate proteoglycan and fibronectin in GC-induced glaucoma (Tawara et al., 2008), suggesting different etiological mechanisms for TM outflow obstruction. A recent study has shown that there is increased basement membrane length underlying the inner wall endothelium of SC in GC-induced glaucoma, and a similar increase in basement membrane length was shown to correlate with decreasing outflow resistance in a mouse model of GC-induced OHT (Overby et al., 2014b). Myofibroblasts are also observed in the JCT in GC-induced glaucoma and are characterized by prominent cytoplasmic filaments, dense regions of the cell membrane likely representing adhesion plaques and an incomplete surrounding basement membrane (Johnson et al., 1997). These myofibroblasts may derive from transformation of TM cells following prolonged GC exposure (Johnson et al., 1997), and may further increase outflow resistance through rho-dependent ECM assembly or cell contractility (Torr et al., 2015). α -smooth muscle actin positive myofibroblasts are also present along the outer wall of SC (Overby et al., 2014b) in mice treated with GC-induced OHT..

Similar ultrastructural changes in the ECM have been described in organ-cultured human eyes perfused with DEX (Clark et al., 1995b). After 12 days of exposure, DEX-responder eyes exhibited thickened trabecular beams, decreased inter-trabecular spaces, and thickened JCT. There was also accumulation of amorphogranular ECM and fibronectin in the JCT and underlying the inner wall of SC, consistent with findings reported from patient tissues mentioned above. In contrast, non-responder eyes treated with DEX appeared morphologically similar to untreated controls, suggesting that the morphological alterations in the TM are closely associated with outflow obstruction in GC-induced OHT (Clark et al., 1995b). Another study showed that DEX treatment increases the ^3H -glucosamine incorporation rate into indigestible glycosaminoglycans (GAGs) after 2-3 weeks (Johnson et al., 1990), and IOP was correlated with total GAG levels in the TM (Johnson and Knepper, 1994). In human TM cells, 500 nM DEX treatment for 24 hrs or 12 days decreases hyaluronic acid synthesis, which may contribute to ECM accumulation if, rather than

functioning as a resistive barrier, hyaluronic acid acts as an inert lining that limits ECM adhesion within inter-trabecular spaces (Engelbrecht-Schnür et al., 1997).

In addition to effects on GAGs mentioned above, GCs modulate the expression and secretion of various ECM proteins. Human TM cells exposed to DEX showed increased expression of laminin (Dickerson et al., 1998; Filla et al., 2014), fibronectin (Steely et al., 1992; Zhou et al., 1998), type IV collagen (Zhou et al., 1998) and elastin (Yun et al., 1989). These proteins may accumulate in the TM to obstruct outflow. DEX also increased thrombospondin-1 expression in human TM cells, which may regulate the behavior of TGF β that influences ECM accumulation in the TM (Flügel-Koch et al., 2004). DEX may also influence the expression of proteolytic enzymes that regulate ECM turn-over, including matrix metalloproteinases and tissue plasminogen activator (tPA) (Samples et al., 1993; Snyder et al., 1993; el-Shabrawi et al., 2000). Recombinant tPA inhibits the IOP elevation induced by prednisolone in sheep (Gerometta et al., 2013; Candia et al., 2014) and the increase in outflow resistance induced by triamcinolone acetonide in mice (Kumar et al., 2013b).

GC-Induced Changes to Cross-Linked Actin Networks (CLANs)

Cross-linked actin networks (CLANs) are cytoskeletal arrangements organized as geodesic dome-like structures or “tangles” of actin filaments. As the cytoskeleton regulates the biomechanical and dynamic behavior of cells and tissues, CLAN formation may alter the biomechanical properties, such as stiffness, of the TM or influence cellular functions such as phagocytosis, contractility or ECM turn-over to affect outflow. Biomechanical alterations may be particularly important in light of recent evidence suggesting that TM and SC cell or tissue stiffness become altered in glaucoma (Last et al., 2011; Camras et al., 2014; Overby et al., 2014c).

CLAN formation increases in response to DEX, as observed in cultured human TM cells (Wilson et al., 1993; Clark et al., 1994) and in perfusion-cultured human TM exposed to DEX (Clark et al., 2005). CLANs are also observed in bovine TM tissue explants exposed to DEX (Wade et al., 2009). Human TM cells isolated from glaucomatous donors have a greater number of basal CLANs and a greater increase in DEX-induced CLAN formation relative to TM cells from normotensive donors (Clark et al., 1995a). CLANs also exist in the TM tissues from both normal and glaucomatous eyes that have not been manipulated by tissue or organ culture, with quantitative estimates predicting that nearly every cell in glaucomatous TM possesses CLANs (Wade et al., 2009).

CLAN formation is particularly robust in bovine TM cells, exhibiting similar dimensions, morphology and half-life as CLANs observed even in the most responsive non-immortalized human TM cells (Wade et al., 2009; Mao et al., 2012). Because bovine TM cells are particularly hardy and can thrive in low serum or serum-free conditions, these cells are well suited to complement studies of CLAN formation in human TM cells. Studies with bovine TM cells have revealed that CLAN formation is stimulated by aqueous humor (Wade et al., 2009), decorin or TGF- β 2 (O'Reilly et al., 2011) in the absence of DEX. Antibody neutralization of TGF- β 2, blockade of the TGF- β 2 receptor, or inhibition of canonical TGF- β 2 signaling through SMAD-3 reduced CLAN formation in bovine TM cells treated with

aqueous humor (O'Reilly et al., 2011). A separate study, however, failed to show find TGF- β 2 induced CLAN formation in human cells from a commercial provider purportedly derived from TM (Yuan et al., 2013).

CLAN formation in human TM cells appears to involve syndecan-4, integrin β 1 and β 3. Syndecan-4 is localized to CLANS that form transiently after re-plating of TM cells (Filla et al., 2006), and a knock-down of syndecan-4 expression reduces this CLAN formation (Filla et al., 2014). Furthermore, DEX increases the deposition of laminin-5, and the syndecan-4 binding peptide derived from laminin-5 induces CLAN formation through a PKC ϵ -dependent signaling pathway (Filla et al., 2014). Immobilized antibodies to β 1 integrin also induced CLAN formation, as did soluble β 3 integrin-activating antibodies in human TM cells (Filla et al., 2006; 2009), and CLANS induced by activation of β 3 integrin appear structurally similar to CLANS induced by DEX (Filla et al., 2011). Genomic and proteomic analysis revealed that several components of the actin cytoskeleton are up-regulated in response to DEX with some of these, such as PDZ and LIM domain (PDLIM1), co-localizing with CLANS in human TM cells (Clark et al., 2013). CLAN formation also appears to be induced by Wnt5a that is up-regulated by 100 nM DEX exposure for 8 days, with signaling partly mediated by the Wnt5a receptor ROR2 (Yuan et al., 2013). CLAN formation has recently been demonstrated in mouse TM cells isolated using a magnetic bead technique and subsequently treated with DEX (Mao et al., 2013).

Although transient CLAN-like structures have been observed in freshly plated cells of various cell types (Lazarides, 1976), persistent CLAN formation that was previously thought to be fairly unique to TM cells (Clark et al., 1994; Clark and Wordinger, 2009) has been observed in other ocular cell types. Schlemm's canal cells from glaucomatous eyes exhibit a disordered and tangled actin cytoskeleton, with structures resembling, albeit not identical to, CLANS (Read et al., 2006). CLANS have also been observed in lamina cribrosa (LC) cells both in tissue culture and in situ explants from human and bovine eyes (Job et al., 2010). As in the TM, CLANS in the LC cells were markedly increased with DEX treatment with larger and more CLANS in LC cells cultured from glaucomatous relative to normotensive donors (Job et al., 2010). It is intriguing that CLAN formation is a common feature of TM and LC cells that are both centrally involved in the pathogenesis of OHT and glaucomatous optic neuropathy. Ongoing studies aim to further clarify the factors regulating CLAN formation and the role of CLANS in the pathogenesis of the TM and LC.

Ratio of GR β /GR α may explain sensitivity of TM cells to GCs

Although the molecular mechanism(s) responsible for differences in susceptibility to GC-induced OHT are not completely known, recent evidence suggests that alternative splicing of the glucocorticoid receptor (GR) into GR α and GR β isoforms may alter the GC response in TM cells (Jain et al., 2014). GR α is a transcription factor that is typically present within the cytoplasm, but GR α translocates to the nucleus when bound to GCs. Nuclear translocation of GR α depends on HSP90 (Zhang et al., 2006) and FKBP51 (Zhang et al., 2008) but not the cytoskeleton in human TM cells (Dibas et al., 2012). In the nucleus, GR α homodimers regulate the expression, either positively or negatively, of various genes that contain GC response elements (GREs). GR β , in contrast, is unable to bind GCs due to

alternative splicing of exon 9 that disrupts the GC-binding domain. GR β maintains its ability to dimerize with GR α , but has reduced transcriptional activity, and GR β therefore acts as a negative regulator of GR α . TM cells express both GR α and GR β , but glaucomatous TM cells appear to have lower GR β compared to TM cells from normotensive individuals (Zhang et al., 2005), which may be related to differential expression of serine-arginine rich proteins (SRPs) that regulate spliceosome activity (Fingert et al., 2010; Jain et al., 2012). The lower GR β / α ratio in glaucomatous TM cells may contribute to greater GC-susceptibility. Alteration of spliceosome activity to increase GR β levels relative to GR α using thailanstatins (Jain et al., 2013) or bombesin peptide (Jain et al., 2012) reduces the GC response in cultured TM cells. Compounds that modulate the GR β / α ratio in TM cells may therefore be promising new therapeutics to treat steroid-induced ocular hypertension as well as glaucoma. We have previously shown that glaucoma TM (GTM) cells have lower amounts of GR β compared to normal TM cells, making GTM cells more sensitive to GCs (Zhang et al., 2005), which may play a role in the pathogenesis of POAG.

Animal Models of GC-OHT

GC-OHT does not only occur in man; experimental studies have reported GC-OHT in 8 other species (Table 2). In most of these studies, the potent GC dexamethasone (DEX) was administered by topical ocular dosing. However, there are some differences in the degree of IOP elevation and the percentage of steroid responders in the treated animals. It should be noted that the aqueous humor outflow anatomy differs among different species, which may explain some of these differences. Also, some species appear to have significant systemic GC effects, despite “local” delivery.

Rabbits

Rabbits were one of the first species besides man to be tested for GC-induced OHT. Lorenzetti administered 4 different GCs by topical ocular drops to rabbits for up to 12 weeks and found concentration dependent increases in IOP in 75% of the treated rabbits as well as systemic side effects at the higher doses (loss in body weight and sometimes death) (Lorenzetti, 1970). Topical administration of dexamethasone 21-phosphate and betamethasone 17-valerate were the most effective GCs in elevating IOP by 5-10 mm Hg as measured by applanation tonometry. This IOP elevation was associated with decreased outflow facility. IOPs returned to normal when topical dosing was discontinued. Topical ocular dosing with potent GCs for 3-5 weeks caused thickening of trabecular beams, loss of TM cells, and increased deposition of extracellular matrix material in the outflow pathway, including at the aqueous plexus (Ticho et al., 1979; François et al., 1984; Knepper et al., 1985; Qin et al., 2010). However, not everyone has been successful in generating ocular hypertension in rabbits by topical ocular administration of potent GCs (Hester et al., 1987; personal communication). Therefore, other routes of administration have been used. Weekly subconjunctival injections of betamethasone (Bonomi et al., 1978; Hester et al., 1987; Melena et al., 1997), cortisone, triamcinolone (Hester et al., 1987) or dexamethasone (Song et al., 2011) reproducibly elevated IOP without the labor of daily topical ocular administration. Intravitreal injections of triamcinolone acetate, either as a suspension or encapsulated in microspheres, also significantly elevated IOP in rabbits for at least 56 days

(Song et al., 2011; Zarei-Ghanavati et al., 2012). In one study, this method of inducing ocular hypertension for 30 days was associated with reduced focal ERG and focal VEP amplitudes and increased latency times, suggesting pressure-induced damage to the retina (Song et al., 2011) or a direct deleterious effect of GCs on the retina.

Cats

There have been several studies demonstrating GC-induced ocular hypertension in cats. Topical ocular administration 2 or 3x daily of 1% dexamethasone phosphate or 1% prednisolone acetate progressively raised IOP without any significant change in body weight (Zhan et al., 1992). IOPs returned to baseline pressures within 6-7 days after discontinuing GC administration. In another study, 5 different GCs were administered by topical ocular dosing 3x/day for 28 days (Bhattacharjee et al., 1999). Dosing with 0.1% dexamethasone or 1% prednisolone acetate significantly elevated IOP by 5 mm Hg starting 5-7 days after administration reaching peak values at 2 weeks. Pressures were measured by pneumotonometry and returned to baseline 7 days after discontinuing therapy. Administration of 1% rimexolone slightly increased IOP, but neither 0.25% fluorometholone nor 0.5% loteprednol etabonate had appreciable effects on IOP.

Dogs

Oral administration of hydrocortisone did not elevate IOP in ocular normotensive dogs (Herring et al., 2004). Intravitreal injection of triamcinolone acetonide (8 mg) did not induce sustained IOP elevation over 3 months of monitoring IOPs (Molleda et al., 2008). However, glaucomatous beagles developed significant ocular hypertension with a 5 mm Hg increase within 7-10 days after topical ocular administration of 0.1% dexamethasone dosed 4x/day (Gelatt and Mackay, 1998). This IOP elevation from baseline was reversed within 7 days after cessation of GC administration.

Cows

Cattle also develop GC-induced OHT. Topical ocular administration of 1% prednisolone acetate 3x/day for 49 days to 3-5 year old female cows doubled IOP to 30 mm Hg as measured by Perkins applanation tonometry starting 3 weeks after therapy and peaked at 4 weeks (Gerometta et al., 2004). 100% of the cattle developed GC-induced OHT, and IOPs returned to baseline values 3 weeks after discontinuation of treatment. These results were confirmed in a second study, which also evaluated GC-induced changes in TM cell gene expression, which may help identify the molecular mechanisms associated with GC-induced ocular hypertension (Danas et al., 2011). Bovine eyes treated with topical 0.5% prednisolone 3x/day for 7 weeks exhibited plaque-like ECM deposits more typical of POAG, in addition to accumulation of basement membrane and fine fibrillar material observed in human eyes with of GC-induced glaucoma (Tektaş et al., 2010). An ex vivo perfusion culture model also confirmed GC-OHT in bovine eyes (Mao et al., 2011). Mao and colleagues set up bovine anterior segments for constant flow, variable pressure perfusion culture and showed significant IOP elevation of 4-10 mm Hg in 40% of the eyes perfused with dexamethasone (100 nM) over the course of 7 days. Myocilin protein was induced in the responder, but not the non-responder eyes, and responder eyes also had increased collagen deposition in the TM. Although the responder rate is similar to that seen

in humans and non-human primates, it is not clear the reason(s) for the discrepancies between the responder rates in the in vivo and ex vivo models.

Sheep

Similar to GC-induced OHT in cows, topical ocular dosing of sheep with 0.5% prednisolone acetate 3x/day significantly increased IOP within 1 week of treatment and peaked with a 16 mm Hg rise at 2 weeks (Gerometta et al., 2009). Again, applanation tonometry was used, and 100% of the animals were steroid responders. IOP returned to baseline levels 1-3 weeks after discontinuation of treatment. This sheep model of GC-induced OHT has been used to test a variety of disease modifying IOP lowering therapies. Transduction of the TM with an adenovirus expression vector that induces matrix metalloproteinase 1 expression under the control of a GRE promoter (Ad5.GRE.MMP1) both prevented GC mediated IOP elevation as well as lowered IOP after induction of GC-OHT (Gerometta et al., 2010).

Subconjunctival injection of the cortisone compound anecortave acetate prevented prednisolone acetate-induced OHT and also lowered IOP in already GC-ocular hypertensive eyes (Candia et al., 2010). Anecortave acetate blocked the GC mediated decrease in the aqueous humor outflow facility, suggesting activity directly on the TM. Either intravitreal (Gerometta et al., 2013) or intracameral (Candia et al., 2014) injection of tPA lowered IOPs of sheep with prednisolone acetate-induced ocular hypertension. IOP lowering by tPA may be mediated by enhancing extracellular matrix turnover in the TM.

Non-human primates

Cynomolgous monkeys were behaviorally trained for conscious IOP measurement by pneumotonometry prior to dosing 0.1% dexamethasone by topical ocular administration 3x/day for 4 weeks (Fingert et al., 2001). Five of the 11 dosed monkeys developed significant IOP elevation of 10 mm Hg starting 7 days after dosing and reaching a maximum at 4 weeks. IOPs returned to baseline levels within 10-14 days of discontinuing dosing. When the study was repeated, the individual monkeys displayed the same IOP response to dexamethasone (they remained GC responders or non-responders). This 40% response rate is similar to the GC-induced OHT response rate seen clinically in man.

Rats

There have been several reports of DEX-induced OHT in rats. Sawaguchi and colleagues administered topical ocular 0.1% DEX 4X/day to young Wistar rats and showed significantly elevated IOP from 2-4 weeks of dosing. IOP was measured using a Tonopen. They reported no change in myocilin gene expression in the TM or Schlemm's canal (Sawaguchi et al., 2005). Proteomics (2-DIGE/MS) experiments were also conducted to look for changes in TM (Shinzato et al., 2007) and retina (Miyara et al., 2008) protein expression. Following 4 weeks of topical DEX treatment, the investigators demonstrated an upregulation of crystallin proteins and a downregulation of collagen type I C-propeptides in the trabecular meshwork (Shinzato et al., 2007). The changes in collagen propeptides seen after chronic DEX treatment in rats may be related to ocular hypertension (Aihara, 2003) and outflow obstruction (Dai et al., 2009) that occurs in mice with type I collagen mutations.

In a recent study, Sprague-Dawley rats received topical ocular 0.1% DEX eyedrops 2x/day for up to 62 days (Razali et al., 2015a). IOPs were elevated beginning 8 days after treatment and increased by 36-43% at days 29-62 in approximately 80% of the treated rats. IOPs returned to normal within a week of discontinuing DEX treatment. This IOP elevation was associated with increased TM thickness and decreased TM cell number as well as decreased thickness and cell counts in the retinal ganglion cell layer. Trans-resveratrol lowered IOP in normotensive rats and rats with steroid-induced ocular hypertension (Razali et al., 2015b). The effects appeared to be mediated through A₁ adenosine receptors, as pretreatment with A₁ antagonist abolished the effect, and the IOP-lowering effects of trans-resveratrol were additive to A₃ and A_{2A} antagonists. This appears to be the first report of GC-induced glaucomatous retinopathy in the rat.

Mouse Models of GC-OHT and Glaucoma

Recent efforts have established the mouse as a model for aqueous humour dynamics, including mice having a similar IOP and AH turn-over time as humans (Aihara et al., 2003). The functional anatomy of the trabecular outflow pathway in mice mimics that observed in human eyes, with a lamellated trabecular meshwork, continuous SC, and ciliary muscle tendons that connect to the inner wall endothelium (Smith et al., 2001; Ko and Tan, 2013; Overby et al., 2014a). Recent efforts have established techniques to measure conventional outflow facility in mice, with the potential to measure all parameters of aqueous humor dynamics within individual mice (Millar et al., 2011). The pharmacological response of outflow facility in mice mimics that observed in humans (Millar et al., 2011; Boussommier-Calleja et al., 2012), and like humans mice appear to lack the washout effect that is observed during experimental perfusion of all non-human eyes, including even living monkeys (Lei et al., 2011). Thus, mice are believed to provide an animal model that captures many aspects of aqueous humor dynamics in humans, including GC-induced OHT and glaucoma.

Whitlock et al. (Whitlock et al., 2010) were the first to demonstrate GC-induced OHT in mice. In that study, DEX was delivered systemically by an osmotic minipump implanted subcutaneously over a period of 4 weeks. Within 1-2 weeks, IOP elevation became statistically significant, reaching an elevation of approximately 3 to 4 mmHg by 3 weeks. IOP was measured using a rebound tonometer calibrated for mice. The elevation in IOP was not attributable to increased blood pressure, and the IOP elevation in response to DEX could be offset by treatment with latrunculin or a rho-kinase inhibitor (Y-39983) that increased conventional outflow facility. Whitlock did not report whether DEX treatment influenced retinal ganglion cells or optic nerve axon loss, but with the relatively modest elevations in IOP, there may be minimal neuropathic damage. The technique using osmotic minipumps to deliver systemic DEX and induce GC-OHT in mice has been reproduced by other groups who reported that the IOP elevation correlated with decreased outflow facility (Overby et al., 2014b) and that IOP may be decreased by cabergoline, a mixed dopamine and serotonin agonist (Platania et al., 2013).

Zode et al. examined GC-induced OHT in mice in response to topical DEX (0.1%) delivered three times per day for up to 6 weeks in C57BL/6 mice (Zode et al., 2014). IOP was elevated by 3.3 mmHg by 2 weeks, and continually increased to 7.7 mmHg at 6 weeks. The IOP

elevation was associated with a significant reduction in pattern electroretinography, loss of retinal ganglion cells and optic nerve axons. GC-induced OHT was associated with ER stress in the TM, evidenced by up-regulation of protein markers GRP78 and CHOP that were apparent within 1 week of DEX exposure prior to significant IOP elevation. DEX treatment also led to an increase in MYOC expression in the anterior segment. Withdrawal of DEX was associated with a recovery of IOP and ER stress markers to baseline. Mice deficient in *Chop* exhibited significantly reduced IOP elevation, GRP78 expression and MYOC expression in response to DEX compared to wild-type mice. Reduction in ER stress by the chemical chaperone sodium 4-phenylbutyrate (PBA) significantly reduced the IOP elevation response to DEX and nearly eliminated the expression of ER stress markers GRP78, XBP-1 and CHOP. PBA also reduced the expression of FN induced by DEX.

Overby et al. (Overby et al., 2014b) measured conventional outflow facility in enucleated eyes from C57BL/6J mice after 4 weeks of systemic DEX delivered by osmotic minipump following the technique originally described by Whitlock (Whitlock et al., 2010). Conventional outflow facility was reduced by approximately 50%, and the reduction in facility was sufficient to account for the majority of the elevation in IOP as predicted by Goldmann's equation. These data suggest that, as in other species, GC-induced OHT in mice arises due to an obstruction in the conventional outflow pathway that reduces outflow facility. GC-induced OHT was associated with accumulation of fine fibrillar and fingerprintlike basement membrane material in the TM that ultrastructurally resembled the ECM deposits reported in human TM specimens with a diagnosis of GC-induced glaucoma (Johnson et al., 1997). Plaque-like sheath deposits were also present in mice treated with systemic DEX, approximating the sheath-derived plaques observed in human POAG (Lütjen-Drecoll et al., 1981). By immunofluorescence, DEX treatment led to an increase in type IV collagen surrounding SC, and by electron microscopy DEX-treatment led to a more dense and continuous basement membrane underlying the inner wall endothelium of SC. The length of basement membrane underlying the inner wall was shown to correlate with decreasing outflow facility and increasing IOP, and a similar increase in basement membrane length was observed after re-examining human TM specimens with a diagnosis of GC-induced glaucoma archived from a prior study (Johnson et al., 1997). DEX treatment was also associated with myofibroblasts observed within the JCT (Johnson et al., 1997) and along the outer wall of SC (Overby et al., 2014b).

Kumar et al. examined the effects of 40 mg/mL triamcinolone acetonide (TA) injected subconjunctivally as a 20 μ L bolus into C57BL/6 mice (Kumar et al., 2013a). After 1 or 3 weeks following the injection, outflow facility measured in enucleated eyes was reduced by approximately 50% compared to naïve or sham-injected control eyes, and results were similar regardless of whether injections were performed unilaterally or bilaterally. Anecortave acetate (AA; 75 mg/mL) injected subconjunctivally as a 20 μ L bolus 2 weeks after TA increased outflow facility back to baseline at the 3 week time point. No significant difference was observed in MYOC expression in response to TA, but AA reduced MYOC expression in the TM. Surprisingly, however, despite the significant reduction in facility, no IOP elevation was reported in response to subconjunctival TA. This puzzling result was attributed to the relatively high proportion of pressure-independent outflow that may occur

in the mouse (Aihara et al., 2003). In a second study by the same group, mice received intracameral injections of adenoviral suspension ($2 \mu\text{L}$ of $3\text{-}4 \times 10^{12}$ virus genomes/mL) carrying sheep *PLAT* (plasminogen activator, tissue) cDNA (AdPLAT) encoding tPA concurrently or 1 week following the subconjunctival injection of TA (Kumar et al., 2013b). Other mice received adenoviral suspension containing no transgene ($2 \mu\text{L}$ of 9×10^{12} vg/mL). Outflow facility was significantly elevated in eyes exhibiting the highest AdPLAT activity, while other eyes that received AdPLAT but with low expression did not differ from eyes receiving TA alone. These data are consistent with other studies in sheep showing intravitreal (Gerometta et al., 2013) or intracameral (Candia et al., 2014) injection of tPA lowered IOPs of sheep with prednisolone acetate-induced ocular hypertension. AdPLAT expression induced up-regulation of PAI-1 (plasminogen activator inhibitor-1), MMP (matrix metalloproteinases) -2, -9 and -13 compared to levels in eyes treated with TA. Presumably, elevated MMP expression contributed to ECM turnover that accompanied the facility increase, with up-regulation of PAI-1 possibly acting as a compensatory mechanism to oppose the elevated expression of proteolytic enzymes. There were no changes in IOP in response to AdPLAT expression.

The three methods of GC delivery used thus far in mice have their own benefits and drawbacks. Topical DEX delivery requires 3x daily eye drops over several weeks by a trained technician, but gives a greater IOP elevation than observed following systemic DEX. This larger IOP elevation likely contributes to the observed optic neuropathy and retinal dysfunction that were not reported in mice treated with systemic DEX. The subconjunctival TA injections appeared to reduce outflow facility after a single injection for up to 3 weeks, but without significant IOP elevation. The minipump delivery method requires surgery, but once implanted delivers DEX over several weeks without technician intervention. A significant limitation of the minipump method is the high drop-out rate (i.e. animals discontinued from study) associated with significant loss of body weight, with a recent study showing nearly a 40% dropout after 3-4 weeks of 3-4 mg/kg/day DEX treatment (Overby et al., 2014b). It may be possible to lower the DEX dosage to reduce the dropout rate while still exhibiting the IOP elevation.

Major Unresolved Issues

GCs cause a plethora of changes to the TM including morphological, cytoskeletal, cell junction, extracellular, and functional changes (Wordinger and Clark, 1999). However, we still do not know which of these effects are responsible for GC-induced OHT. GCs alter the expression of hundreds of genes in the TM, only a subset of which are likely responsible to impairing the outflow facility and elevating IOP. Performing molecular studies on GC responder versus non-responder animals given the same dosing regimen will help address the mechanisms responsible for GC-induced OHT. We currently are using this approach by conducting these studies in the ex vivo bovine perfusion culture model.

Not all subjects receiving the same GC dosing strategy develop GC-induced OHT. This is true in man and in several of the animal models of G-OHT. What is the molecular mechanism(s) responsible for this altered susceptibility to GCs? A potential explanation is the ratio of alternatively spliced isoforms of the glucocorticoid receptor (Jain et al., 2014). A

higher ratio of GR β to GR α appears to make TM cells more resistant to GCs (Zhang et al., 2005, 2007, 2008). Additional studies are required to determine whether this alternatively spliced isoform plays a significant role in determining the development of GC-OHT in vivo.

As we have reviewed, there are 9 species including man that develop GC-induced OHT. However, not all models have been shown to develop GC-induced glaucoma (i.e. damage to retinal ganglion cells, optic nerve, and resulting loss of visual function). Although this now has been demonstrated in mice (Zode et al., 2014), studies in other species should be conducted.

GC-induced OHT is an important clinical problem, especially now with the use of intravitreal GC therapies. Currently, these patients are treated with conventional glaucoma IOP lowering pharmaceutical or surgical therapies. Several new disease modifying therapies have been shown to be effective in animal models of GC-OHT and additional therapeutic approaches could be tested in these models in order to specifically treat this important form of secondary glaucoma.

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Highlights

- Clinical glucocorticoid therapy can cause ocular hypertension and iatrogenic open-angle glaucoma
- Glucocorticoid-induced ocular hypertension occurs in 8 other species, from mice to nonhuman primates
- Animal models of glucocorticoid-induced ocular hypertension and glaucoma are being used to better understand this important side effect of glucocorticoid therapy

Table 1**Side Effects of GC Therapy**

-
- Adrenal insufficiency
 - Osteoporosis
 - Hyperglycemia
 - Leukopenia
 - Weakness and reduced skeletal muscle mass
 - Increased skin fragility & bruising
 - Cushing's syndrome habitus
 - Weight gain and central obesity
 - Delayed puberty
 - Ocular
 - Posterior subcapsular cataracts
 - Ocular hypertension (OHT) and iatrogenic open-angle glaucoma
-

Table 2

Summary of Animal Models of GC-Induced OHT and Glaucoma

Species	GC	Admin Route	OHT	RR	Glaucoma	Systemic Effect
Mouse	DEX	Systemic (OMP)	Yes	100%	NR	Yes
	DEX	Topical Ocular	Yes	100%	Yes	NR
Rat	DEX	Topical Ocular	Yes	NR	NR	Yes
Rabbit	DEX-PO4	Topical Ocular	Yes	75%	NR	Yes
	BM-17-V	Topical Ocular	Yes	75%	NR	Yes
	BM	Subconj Injection	Yes	NR	NR	NR
	DEX	Subconj Injection	Yes	NR	NR	NR
	TA	Ivt Injection	Yes	NR	Yes	NR
Cat	DEX	Topical Ocular	Yes	NR	NR	NR
	DEX-PO4	Topical Ocular	Yes	NR	NR	NR
	Pred-Ac	Topical Ocular	Yes	NR	NR	NR
Dog	DEX	Topical Ocular	Yes	NR	NR	NR
Cow	Pred-Ac	Topical Ocular	Yes	100%	NR	NR
	DEX	POC	Yes	40%	No	No
Sheep	Pred-Ac	Topical Ocular	Yes	100%	NR	NR
NHP	DEX	Topical Ocular	Yes	40%	NR	NR

Abbreviations: BM= betamethasone; BM-17-V = betamethasone 17-valerate; DEX = dexamethasone; DEX-PO4 = dexamethasone 21-phosphate; Ivt = intravitreal; NHP = nonhuman primate; NR = not reported; OMP = osmotic minipump; POC = perfusion organ culture; RR = responder rate; Pred-Ac = prednisolone 21-acetate; Subconj = subconjunctival; TA = triamcinolone acetonide;