Experimental Eye Research 141 (2015) 15–22

Contents lists available at ScienceDirect

Experimental Eye Research

journal homepage: www.elsevier.com/locate/yexer

Animal models of glucocorticoid-induced glaucoma

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ARTICLE INFO

Article history:
Received 13 April 2015
Received in revised form
2 June 2015
Accepted in revised form 3 June 2015
Available online 4 June 2015

Keywords:
Glucocorticoid
Ocular hypertension
Glaucoma
Intraocular pressure
Steroid glaucoma

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.

1. 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, 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).

2. 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

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http://dx.doi.org/10.1016/j.exer.2015.06.002
0014-4835/

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

studies in the 1960s, individuals were given topical ocular dexamethasone or betamethasone (0.1% 3–4×/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 Chevrette, 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).

3. 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.

3.1. 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 ^{3}H-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 h 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 TGFβ 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).

3.2. 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