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Research article

Model systems for the study of steroid-induced IOP elevation

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ABSTRACT

Steroid-induced IOP elevation affects a significant number of patients. It results from a decrease in outflow facility of the aqueous humor. To understand the pathophysiology of this condition a number of model systems have been created. These include ex-vivo cell and organ cultures as well as in-vivo animal models in organisms ranging from rodents to primates. These model systems can be used to investigate specific aspects of steroid-induced IOP elevation. This brief review summarizes the strengths and limitations of the various model systems and provides examples of where these systems have been successfully used to advance our understanding of steroid-induced IOP elevation.

1. Introduction

Steroid-induced ocular hypertension is a frequent complication of chronic treatment with corticosteroids (Jones and Rhee, 2006). Although usually reversible with steroid discontinuation it has been increasingly prevalent because of the use of potent corticosteroids in or around the eye as treatment for various eye diseases (Kiddee et al., 2013). Steroid-induced IOP elevation occurs within weeks in susceptible individuals and is dependent on steroid potency, pharmacokinetics, duration of treatment and route of administration (Becker and Mills, 1963). Approximately one in three individuals on chronic topical steroid therapy will experience IOP elevation and in ~20% of those, IOP elevation will be significant (Becker and Mills, 1963). Intraocular steroids increase the risk for IOP elevation dramatically. In trials of sustained release fluocinolone acetonide implant, over 75% of patients receiving the steroid intravitreally required IOP lowering therapy and 40% required surgical intervention for IOP control (Goldstein et al., 2007). The prevalence of steroid–induced IOP elevation is even higher among patients with glaucoma. Approximately 90% of these patients and 30% of glaucoma suspects will develop moderate IOP elevation after 4 weeks of treatment with steroid drops (Armaly, 1963b).

If unrecognized, prolonged steroid-induced IOP elevation can lead to (or exacerbate) glaucomatous optic neuropathy (Goldmann, 1966) and result in loss of vision. Although it is well established that steroid-induced IOP elevation is the result of increased aqueous humor outflow resistance (Armaly, 1963a; Bernstein and Schwartz, 1962) the exact pathogenetic mechanism remains unknown. However, the similarities in the pathology and the increased prevalence with primary OAG (POAG) suggest that common mechanisms are operational in both conditions.

The rise in IOP caused by steroids is often attributed to alterations in cell cytoskeletal dynamics, and a dysregulation in extracellular matrix (ECM) deposition and remodeling (Clark et al., 1994; Jones and Rhee, 2006; Raghunathan et al., 2015) which result in morphological changes (Tektas and Lutjen-Drecoll, 2009) and reduction in facility of outflow. To try to understand changes at the cellular and molecular level that lead to steroid induced ocular hypertension, a number of ex-vivo and in vivo models for the condition have been created. These model systems have to date provided relevant information but many details of the pathophysiology of this condition still remain unclear. This review summarizes some of the work performed in the various steroid-induced IOP elevation models, highlights some of the strengths and limitations of each one and provides some examples of successful application of model systems in addressing relevant questions.

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2. Ex-vivo model systems

2.1. Tissue culture models

Some of the earliest attempts to understand steroid-induced IOP elevation at the cellular and molecular level led to the isolation of primary trabecular meshwork (TM) cells (Polansky et al., 1979). These cells can be easily isolated from both human post-mortem eyes as well as animal eyes (Crean et al., 1986; Grierson et al., 1985; Yue et al., 1988) and can be used to study the physiology (Clark and Wordinger, 2009; Gasiorowski and Russell, 2009) and gene expression (Paylakhi et al., 2012; Rozsa et al., 2006) of the cells that in large part control ECM deposition and either directly or indirectly ultimately affect outflow facility. The use of TM cells has resulted in some spectacular success stories in the quest to identify genes related to glaucoma. Most notably work on HTM cultures identified the protein encoded by the first glaucoma gene: TIGR/myocilin (Polansky et al., 1997; Stone et al., 1997). Other relevant important findings that come from work on TM cultures treated with steroids include the increased stiffness in trabecular meshwork (McKee et al., 2011), the activation and proliferation of the endoplasmic reticulum and Golgi apparatus and increased extracellular matrix (ECM) deposition (Wilson et al., 1993), the increase in fusion vesicles on TM cell membranes (McCartney et al., 2006), the increase in HTM cell size (Tripathi et al., 1989), the changes in TM cell cytoskeleton (Wilson et al., 1993) and the decreases in phagocytotic activity (Yang and Li, 1996; Zhang et al., 2007), as well as cell migration and proliferation (Clark et al., 1994).

Although transformed lines of TM cells were generated and used in the 90s (Liu et al., 2002; Pang et al., 1994; Tamm et al., 1999) they have fallen out of favor as they seem to deviate significantly in their physiology and gene expression from primary cells (Liu et al., 2002). In fact, even primary cells appear to senesce and change as they progress through different passages (Schachtschabel and Binninger, 1990). The number of viable usable passages depends on donor age with cells from older individuals having decreased replicative capacity. Some TM cultures stop dividing after only a few passages. Typically, primary TM cells grown in culture reflect the physiology of TM cells in vivo only up to passage five. Use of cells beyond this passage may potentially provide unreliable results and should be avoided. Because isolation of TM cells involves dissection of tissue and can potentially result in contamination with other cells from surrounding tissues and because of gene expression changes with passaging, it is advisable to verify the identity of TM cells in primary culture. Most investigators rely for such verification on confirmation of expression of a panel of genes and/or their respective proteins that are typically expressed in TM cells. Prominent among them are myocilin (MYOC), matrix protein Gla (MGP), caveolin 1 (Cav1), collagen 4 alpha 5 (Col4A5) and tissue inhibitor of metalloproteinase 3 (TIMP3) (Du et al., 2012; Hernandez et al., 1987; Kuehn et al., 2011; Mao et al., 2012; Ueda et al., 2000; Xue et al., 2006). Other cell markers include αβ-Crystallin (Welge-Lussen et al., 1999), tissue plasminogen activator (Seftor et al., 1994) and smooth muscle actin (Pang et al., 1994). Significant upregulation of MYOC by steroid treatment is considered also to be strongly indicative of the TM nature of human cells in culture (Polansky et al., 2000) although MYOC mRNA transcription upregulation occurs 12–24 h after exposure to steroids (Joe et al., 2011). An important caveat is that myocilin upregulation (which is universal in humans) does not occur in a number of animal species (e.g. (Sawaguchi et al., 2005)). Since TM cells have avid phagocytic activity (Rohen and van der Zypen, 1968), a phagocytosis assay can also be used as further confirmatory proof that cells in culture are TM cells. In addition contractility assays have been recently developed to characterize and positively identify HTM cells (Dismuke et al., 2014).

Isolation and nutritional requirements of TM cells have been well described (Polansky et al., 1979; Stamer et al., 1998) and involve relatively straightforward manipulations and methods. A number of investigators use rings of tissue left over after keratoplasty to isolate these cells from human post-mortem eyes. If using such a tissue source it is important to thoroughly rinse the tissue with culture medium prior to putting tissue fragments into culture.

The recent development of differentiated HTM cells from stem cells (Abu-Hassan et al., 2015; Ding et al., 2014; Du et al., 2012) opens up the possibility of generating large amounts of TM cells in culture that will be identical and can be used for experimentation (provided they express all relevant markers and behave similarly to primary HTM cells). This development would make generation of cells that can be readily shared between labs a reality. In addition it holds the promise of allowing generation of disease specific cells.

The other important cellular component of the outflow pathways in the area of the juxtacanalicular tissue (JCT) are Schlemm's canal (SC) cells. Contrary to TM cells that are fairly easy to culture, SC cells are notoriously hard to obtain in culture (Dautriche et al., 2014). The method most often used for generating such primary cultures involves the placement of a suture in the SC and lengthy incubations (Stamer et al., 1998). Verification of the nature of the cells involves screening for a panel of markers that are normally expressed on these cells including fibulin-2, VE-Cadherin, integrin-α6 (Perkumas and Stamer, 2012) and PECAM-1 (Dautriche et al., 2015) and the absence of LYVE-1 expression (van der Merwe and Kidson, 2014). SC cells in culture also change their gene and protein expression over time (Lei et al., 2014a, b). Furthermore, Human Schlemm's canal (HSC) cells in traditional culture lose spatial, mechanical, and biochemical cues resulting in altered gene expression and cell signaling compared to cells in-vivo (Dautriche et al., 2015). This in combination with the difficulty in isolating them creates a bottleneck in terms of experimentation with these cells. To date, generation of SC cells from stem cells has not been reported.

Culturing TM and SC cells allows for the observation and study of structural characteristics, biological properties, as well as the growth patterns of the cells (Dautriche et al., 2014). Gene and protein expression after exposure to steroids have been reported (for examples see (Bollinger et al., 2011, 2012; Zhao et al., 2004)) and contribute to our current understanding of some of the processes that lead to steroid-induced IOP elevation. In addition TM and SC cells in cultures have been used to study the biomechanical properties of these cells (Raghunathan et al., 2015). However, use of TM and SC cells in traditional culture to study steroid induced IOP elevation is limited by the lack of a physiologic parameter that one can monitor to indicate functional effects on outflow facility. Thus, even though traditionally cultured TM and SC cells can be used to dissect molecular pathways in these cells, relationship of these pathways to IOP control has to be established in other models.

Notwithstanding the above caveats, traditional cultures of TM cells and SC cells have been used as systems to study steroid-induced IOP elevation. Cells have been treated with a number of steroids including dexamethasone, triamcinolone, prednisolone, cortisol, progesterone, cortexolone, methyltestosterone, fluorometholone, and rimexolone (for examples see (Clark et al., 1994; Raghunathan et al., 2015; Sharma et al., 2014; Sohn et al., 2010)).

It appears that drug partitioning in the TM increases as lipophilicity increases. The rank order of lipophilicity and subsequently observed steroid partitioning was triamcinolone < prednisolone < dexamethasone < triamcinolone acetonide < fluocinolone acetonide < budesonide (Thakur et al., 2011). Although the effects on gene and protein expression are generally similar, the various steroids should not necessarily be considered interchangeable in their

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