The Effects of Dexamethasone on Fibronectin Expression in Cultured Human Trabecular Meshwork Cells

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Topical administration of glucocorticoids to the eye can lead to the development of ocular hypertension. This increase in intraocular pressure is caused by the heightened resistance to flow of aqueous humor from the eye, presumably at the trabecular meshwork (TM). This study reports the effects of dexamethasone (DEX) on the expression of the extracellular matrix protein fibronectin (FN) in cultured human TM cells (HTM). The expression of FN was evaluated in four HTM cell strains by epifluorescence microscopy and immunoblotting and autoradiography of electrophoretically separated cell proteins. There was a heterogeneous response of the four cell strains tested. Treatment of cell strain HTM4 with DEX (10^{-7} mol/L) for 17 d caused an approximate doubling of cell-associated and secreted FN. This DEX-induced increase in FN expression was progressive after the first 7 d of treatment and was blocked partially with a glucocorticoid antagonist, cortexolone. By contrast, DEX treatment induced an intermediate 50-60% increase in FN expression in cell strains HTM10 and HTM2; in HTM6, FN was unchanged after exposure to the glucocorticoid. This model system may be useful to examine molecular changes associated with corticosteroid-induced ocular hypertension and evaluate glaucomatous changes in the TM because increased FN deposition occurs in the aqueous humor outflow pathway of patients with open-angle glaucoma. Invest Ophthalmol Vis Sci 33:2242-2250, 1992

Systemically or topically administered glucocorticoids can effect ocular hypertension and induce glaucoma.1^-6 The anatomic and functional characteristics of steroid-induced glaucoma are similar to clinical features associated with primary open-angle (simple) glaucoma.6^-5 For example, in both types of glaucoma, the iridocorneal angle remains patent, and both diseases are accompanied by a painless rise in intraocular pressure, caused by a decrease in outflow facility.6^-4 A fundamental site of action for the decrease in aqueous outflow, in both diseases, generally is believed to be the trabecular meshwork (TM),6^-8 which contains glucocorticoid receptors.9^-10 Specifically, the mechanism of diminished outflow is attributed to the accumulation of extracellular matrix (ECM)-like material in the juxtacanalicular region of the TM.7^-8 Deposition of this extracellular macromolecular material restricts transcellular outflow of the aqueous and/or prevents normal stretching of the TM by the ciliary muscle.7 Finally, both steroid-induced and open-angle glaucoma cause both quantitative and qualitative changes in the glycosaminoglycan (GAG) content of the TM.11^-13 Thus, in a number of ways, steroid-induced glaucoma can serve as a functional model of simple glaucoma.

Although GAG expression in the TM is changed in the presence of glucocorticoids, this change is only one result of a number of corticosteroid-induced metabolic alterations in the TM. For example, in 1975, it was hypothesized that corticosteroids might act to decrease the phagocytic capabilities of the TM.14 This was proved later to be correct for cultured TM cells.15 Moreover, exposure to the glucocorticoid dexamethasone (DEX) has been shown to inhibit, dramatically, both the migration and proliferation of TM cells.16 This drug also decreases the expression of mature collagens, which are necessary for the maintenance and remodeling of the trabecular beams.17^-18 Exposure of TM cell strains to DEX can modify the secretion of some glycoproteins, especially a group of sialylated proteins which are postulated to be associated with glaucoma.19^-22 These examples illustrate the intimate nature of the interaction between functional TM cells and the secreted ECM and the sensitivity of this interaction to glucocorticoids.

Among the more important glycoproteins secreted by the TM which comprise its ECM is fibronectin (FN).19^-23 This is a large heterodimeric molecule (ap-
proximately, 450 kD or 250 kD in monomeric form) that can be incorporated into the ECM in the form of cross-linked multimolecular fibrils. Two forms of FN were relevant in our study: (1) cell-associated FN (cFN) and (2) plasma FN, which is produced by the liver. The former is synthesized by cells and initially binds to integrin, the FN receptor, before assembly into fibrils. We report our results of studies on FN expression in four different human TM (HTM) cell strains exposed to the potent glucocorticoid DEX (10^-7 mol/l) for 0–42 d.

Materials and Methods

Cell Culture

Three HTM cell strains (HTM4, HTM6, and HTM10) from 18-, 73-, and 54-year-old normal donor eyes (respectively) and a fourth cell strain from a glaucomatous 72-year-old donor eye were propagated in the literature. The TM was dissected from the eyes less than 24 hr postmortem and placed in a single well of a 24-well plate containing Ham's F10 media (Hazelton, St. Lenexa, KS) supplemented with fetal bovine serum 10% (FBS; Hazelton or HyClone [Logan, UT]), L-glutamine (0.292 mg/ml), penicillin (100 units/ml), and streptomycin (0.100 mg/ml). The explanted tissue was incubated at 37°C under a humidified atmosphere containing CO2 7% and air 93% until the cells reached confluence. The four cell strains propagated in this manner grew in monolayers as flat closely packed cells with overlapping cell borders. The cells expressed collagen types I, II, and IV, FN, and laminin (as determined by indirect immunofluorescence analysis). These cells therefore were judged to be equivalent to the cells expressing TM morphologic and antigenic determinants as described in the literature.

The HTM cells were passaged as follows. Cytodex beads (C-3275; Sigma, St. Louis, MO) in a 2% suspension in sterile Dulbecco's phosphate-buffered saline (PBS) were added to confluent HTM monolayers. After approximately 1 wk, gentle washing of the beads with a stream of media from a Pasteur pipette dislodged the cell-covered beads from the confluent cultures. Subsequently, the dislodged beads were aspirated and transferred to new plates and the cells on the original plate were left to regrow as before. The passage of cells using Cytodex beads allowed much longer retention of "normal" TM morphology than did standard passage by trypsinization. For experimental testing, the cells were grown in six-well plates and allowed to reach confluence for at least 1 wk before dosing began.

We dissolved DEX (10^-4 mol/l) in ethanol and diluted it 1:1000 in culture media to expose cells to a final concentration of 10^-7 mol/l. Control media received comparable levels of ethanol alone. The cells were treated for 0–42 d. On the last day of each experiment, the media was switched from enriched Ham's F-10 to RPMI-1640 (Selectamine; Gibco, Grand Island, NY), containing no 1H-L-leucine but supplemented with FBS 2%, antibiotics, with and without 10^-7 mol/l DEX, and 4,5-3H-leucine at a final concentration of 10 μCi/ml. To facilitate later quantitation of FN content of the media, exactly 5.0 ml of radiolabeled RPMI media was added to each confluent well. An experiment also was done to examine the effects of a glucocorticoid antagonist, cortisolone (Reichstein's substance S), on the DEX-induced modification of HTM4 cellular FN expression. For this experiment, the wells of a six-well plate were untreated or treated for 17 d with 10^-7 mol/l DEX, with 10^-6 mol/l cortisolone, or with both drugs.

Immunofluorescent Microscopy

The HTM cells were grown to confluence on 9 x 9-mm cover slips in 24-well plates. After 14–21 d of control or DEX (10^-7 mol/l) treatment, the cells were processed for indirect immunofluorescence analysis using a monoclonal anti-human FN primary antibody (MAB042; Chemicon, Temecula, CA) and a fluorescein isothiocyanate-conjugated goat anti-mouse secondary antibody (602-25; Kappel, Malvern, PA). The FN was visualized by epifluorescence using a Nikon (Garden City, NY) photomicroscope with Kodak 800 ASA film (Eastman Kodak, Rochester, NY).

Electrophoresis and Immunoblotting

After removal and subsequent storage of the media (−20°C), each well of the six-well plate was washed three times with 1.0 ml of cold PBS to remove nonspecifically bound 1H-leucine. The radiolabeled cells were harvested by scraping with a rubber policeman (total volume, 1.5 ml of PBS) and pelleted by centrifugation at 12,000 X g for 10 min at 700 X g. The TM cell-associated proteins were extracted from the pellet by adding 250 μl of 9 mol/l urea solution containing Nonidet P-40 2% (Sigma, St. Louis, MO). The mixture then was allowed to incubate with periodic gentle stirring for 1 hr at room temperature. After double centrifugation at 12,000 X g for 10 min, the final supernatant was aspirated and aliquoted into 50-μl portions for storage at −80°C. The protein content of extract from each well was determined by linear regression using the bicinchoninic acid assay (Pierce, Rockford, IL) with ovalbumin (1–25 μg) as the protein standard. Two 10-μl samples of extract from each well also underwent radioassays for total tritium activity using an LKB (Washington, DC) 1219 scintillation counter. The percentage of trichloroacetic acid-precipitable radiolabeled material was always greater.
than 97%, indicating excellent incorporation of \(^3\)H-leucine into total cellular protein.

The TM samples extracted in urea and Nonidet underwent electrophoresis in polyacrylamide gels (6% total, 5% cross linker), using minor modifications of the Laemmli\(^{30}\) method. After addition of an equal volume of 2X sample buffer and denaturation at 95°C for 5 min, the samples (containing 10–18 \(\mu\)g of protein) underwent electrophoresis at a constant current of 40 mA at 4°C. Although sodium dodecyl sulfate (SDS) was present in both the sample and running buffers, none was added to either the stacking or resolving gels because its absence reportedly adds to the resolution of proteins in acrylamide gel matrices.\(^{31}\) Both molecular weight (Biorad, Richmond, CA, or Amersham, Arlington Heights, IL) and human plasma FN (Calbiochem, La Jolla, CA) standards were included on each gel. After fixation in methanol 50% and acetic acid 10%, the gels were stained with either Coomassie brilliant blue (Sigma) or Gelcode silver stain (Pierce).\(^{29}\) Protein banding on each gel was quantified in terms of the percentage of total area by protein loaded onto each gel slot. The results from the protein loaded. The results of a single experiment in which DEX induced a doubling of the amount of FN over that detected in control samples is shown in Figure 3A. The mean glucocorticoid-induced increase in the amount of FN on each electrophorogram (Fig. 2). The experimental results were evaluated in terms of immunodetected FN compared with the amount of total protein loaded onto each gel slot. The results from the cFN immunodetection experiments and from those dealing with secreted FN in media were compared for statistical significance using paired student t-tests.

Media from each well also were examined for FN content by electrophoresis in 6% gels, using two different methods. First, 0.5 ml of media from each confluent well was precipitated by adding 0.167 ml of trichloroacetic acid 40% for 1 hr at 4°C. After sequential washing with cold diethyl ether–ethanol and ethanol, the precipitate was solubilized by adding 100 \(\mu\)l of 2X Laemmli sample buffer with heating and sonication. The solubilized samples underwent electrophoresis on gels and were immunoblotted as before. The results of each experiment were evaluated subsequently as the total amount (in micrograms) of FN per 0.5 ml of media. A second autofluorographic method was used to detect newly synthesized FN. Samples of media (0.3 ml) were precipitated with trichloroacetic acid and underwent electrophoresis as before. After overnight fixation, the gels were washed for 30 min in distilled deionized water and soaked for 20 min in 1 mol/l sodium salicylate. The gels were dried on filter paper and exposed to Kodak XAR film at -80°C for 3–4 d. Autofluorograms were scanned in two dimensions using the laser densitometer to quantify the bands corresponding to the molecular weight of the FN monomer (approximately 250 kD). A comparison of control specimens to DEX-treated samples was made in arbitrary units per 0.3 ml of media.

**Results**

**Epifluorescent Microscopy**

The extracellular expression of FN in control and DEX-treated HTM4 cell strains was typical (Fig. 1). For control cells, FN expression was seen as lightly stained loosely aligned filaments on the cell surface or as large bundles generally set at the periphery of individual cells. After 21 d of DEX treatment, there appeared to be increased expression of FN associated with the cell surface and, in addition, accumulation of large FN bundles.

**Electrophoretic Detection of FN in Strain HTM4**

*Western blotting of cell extracts:* Principally, immunodetection of electrophoretically blotted HTM4 proteins from 6% gels was used to show that FN expression was increased by DEX treatment of cells. A representative FN standard curve (integrated volume versus nanograms of plasma FN) was used to quantify the amount of FN on each electroblot (Fig. 2). The results of a single experiment in which DEX induced a doubling of the amount of FN over that detected in control samples is shown in Figure 3A. The mean glucocorticoid-induced increase in cFN was 197% ± 32% of that of control (\(n = 7, P < 0.002\)).
Western blotting and autofluorography of secreted proteins: Electrophoresis of trichloroacetic acid-precipitated cell-free media was used to determine if the DEX-stimulated increase in cFN was reflected in FN secretion by the cells into the media. Figure 3B shows a representative experiment; a twofold increase of FN was found in the media from DEX-treated HTM cells compared with media from control cells. Immunodetected FN in DEX-treated media averaged 203% ± 75% of that of FN in control media \((n = 5, P < 0.005)\). The relatively large standard error of the mean for media FN determinations was caused probably by: (1) the difficulty in completely solubilizing the precipitates in Laemmli sample buffer before gel electrophoresis and (2) the mechanical destruction of the gel caused by the large amount of serum albumin present in the samples.

Autofluorography of the precipitated media containing \(^{3}H\)-labeled proteins in 6% polyacrylamide gels showed well-separated banding in the region of the gel corresponding to FN (approximately 250 kD). A representative autofluorogram is shown in Figure 4A. Integration of the FN bands from a representative fluorograph is shown in Figure 4B. The average area integrated in the FN (250 kD) band of DEX-treated
Fig. 3. A composite panel showing the results of one experiment for dexamethasone-induced increases in the fibronectin associated with protein extracts of HTM4 cells (a) and media (b). Error bar represents the SD for three control wells and three DEX-treated wells.

samples was 271% ± 124% of the area integrated in control samples (n = 5, P < 0.001).

Polyacrylamide gel electrophoresis of cell extracts: One-dimensional electrophoresis of HTM4 cell extracts in polyacrylamide 6% gels showed a marked increase in a protein band in the high molecular weight region of the gels. This band virtually comigrated with the plasma FN standard (approximately 250 kD). Figure 5 demonstrates the densitometric traces of FN isolated from control and DEX-treated TM cells. In two different experiments, integration of the FN band showed a DEX-induced increase in the percentage of total area for either silver (152% ± 28.3% of control) or Coomassie blue staining (201% ± 53% of control). Thus, the results for standard electrophoresis of total cell-associated proteins repeat and reinforce our results for immunodetected FN intimately associated with the TM cell. The disparity between the silver and Coomassie blue staining reflects either the nonlinearity of silver staining or the detection of nucleic acid in the silver-stained gels. Coomassie blue does not stain nucleic acids.

Time-course development of DEX-induced FN effects: The DEX-mediated onset of increased FN deposition in HTM4 cells was examined over the course of 21 d of treatment using electrophoresis and immunodetection of FN in cell extracts as described. There was a lag period of approximately 7 d before the onset of a DEX-stimulated increase in cFN deposition (Fig. 6). After 7 d of DEX treatment, there was a progressive time-dependent increase in FN expression. By day 21 of DEX exposure, the percentage of total urea-soluble proteins attributable to cFN had increased by over 3.5-fold that of day 0 values (from 0.58-2.19%). A second experiment determined whether the FN increase was reversible after removal of the glucocorticoid. Forty-two days of DEX exposure caused an 8.5-fold increase in TM cFN (Figs. 7A, 7C). There did not appear to be an effect of DEX withdrawal on FN expression. The amount of cFN after 21 d of DEX expo-
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Fig. 5. Scanning densitometry of top one-third of silver-stained, 6% polyacrylamide gels using protein extracts from HTM4 cells after 17 days of either no treatment (dotted line) or treatment with $10^{-7}$ M dexamethasone (solid line).

Effect of a glucocorticoid antagonist on DEX-induced FN results: Concomitant exposure of HTM4 cells with the glucocorticoid antagonist cortexolone ($10^{-6}$ mol/l) and $10^{-7}$ mol/l DEX led to a partial inhibition of the DEX-induced increase in cFN (control FN, 1.36%; DEX-induced FN, 3.22%; DEX-induced plus cortexolone FN, 2.00%). There was no difference in the amount of FN expressed between control cells and those exposed to cortexolone alone. Thus, partial, but specific, blockade of the DEX-induced increase in HTM FN expression occurred by using the glucocorticoid receptor antagonist.

The Effects of Corticosteroid Exposure on Other HTM Cell Strains

Besides cell strain HTM4, three other cultured TM cell strains (HTM6, HTM10, and HTM2) were tested for sensitivity to 17 d of DEX exposure. By contrast with the HTM4 cell strain, there was no DEX-induced alteration of FN expression in the HTM6 cell strain (Fig. 8A). However, the basal (control) level of FN expression was significantly higher in HTM6 cells than in cells of the HTM4 strain. In addition, HTM4 usually (70% of all experiments) expressed FN at 0.5–1% of total urea-soluble protein; the percentage of FN in HTM6 urea-soluble proteins was approximately 1.6–2%.

Both HTM10 and HTM2 cells showed a FN response to $10^{-7}$ mol/l DEX exposure that was intermediate (respectively, 151% and 158% of control) to that of HTM4 and HTM6 (Figs. 8B–C). Moreover, the secretion of FN into the media of HTM10 cells also increased by approximately 65% after 17 d of DEX treatment (results not shown). The basal level of cFN expression in HTM10 was close to that of HTM4, representing 0.5–0.7% of total urea-soluble proteins. The basal FN values for HTM2 cells was closer to that of HTM6 (1.3% of total urea-soluble protein).

Discussion

We documented quantitatively the differential expression of the ECM glycoprotein FN in four different cultured HTM cell strains, both in the absence and the presence of a potent corticosteroid. The cell strain HTM4 showed a dramatic increase in FN deposition after stimulation with $10^{-7}$ mol/l DEX. This change in both the quantity and organization of FN was found initially by using epifluorescent microscopy.

An essentially twofold increase in FN synthesis in HTM4 cells exposed to DEX for 17 d was documented further by electrophoretic methods including
Fig. 7. A single experiment evaluating the effect of removal of dexamethasone on the DEX-induced increase in fibronectin from HTM4 cells. The cells were treated as follows: A: control—42 days of no dexamethasone (DEX) treatment; B was 21 days of no steroid followed by 21 days of 10⁻⁷ M DEX; C: 42 days of continuous exposure to DEX; and D: 21 days of exposure to DEX, then 21 days of no dexamethasone.

...western blotting of the cell extracts and media, autoradiography, and polyacrylamide 6% gel electrophoresis of total cellular proteins (Table 1). Specifically, DEX treatment increased the expression of both cFN and secreted FN in HTM4 cells; however, it was necessary to ensure that the FN was newly synthesized protein and not plasma FN derived from the FBS in the media. (Plasma FN incorporation into cellular FN fibrils has been demonstrated previously. 34) Therefore, autoradiography was used to show that the radiometric intensity of the 3H-labeled FN bands comigrating with FN standards also increased after exposure to DEX. Time-course studies of the DEX-induced change in HTM4 FN expression showed that augmentation of cFN did not begin until approximately day 7 of exposure to the glucocorticoid. After this lag period, there was a significant, progressive increase in FN deposition. A second time-course experiment showed that the DEX-induced FN increase was not reversed to basal levels by removing the steroid. This result agreed with a previous report on DEX-induced changes in FN expression in human fibrosarcoma cells. 35 Finally, the DEX-induced increase in FN appeared to be mediated through the glucocorticoid receptor because the corticosteroid antagonist cortexolone partially antagonized the response.

...FN-based response of different HTM cell strains may reflect the diverse in vivo responsiveness (ie, induction of ocular hypertension) in the human population to topical application of glucocorticoids. 1-6 A glucocorticoid-induced increase in cFN synthesis and secretion previously was found in other cell strains, such as normal human fibroblasts, human fibrosarcomas, and rat hepatomas. 35,36 In normal fibroblasts, treatment with DEX resulted in a twofold increase in FN biosynthesis. 35 Other studies involving the effects of DEX on HTM cell strains have shown diverse results. For example, by using indirect immunofluorescence, a net decrease in the FN staining of...
cultured TM cells was found after 2 wk of exposure to $10^{-7}$ mol/l DEX. Others reported that DEX treatment of TM cells induced a slight reduction in FN messenger RNA synthesis and a substantial increase in stromelysin messenger RNA synthesis. Because stromelysin is a metalloprotease that degrades FN extracellularly, an increase in its rate of synthesis might be expected to result in an overall reduction of FN. Finally, recently it was reported that HTM cell stromelysin production was inhibited in a dose-dependent manner by glucocorticoids. This result might be expected to lead to a net increase in FN content.

It is not surprising that the results of different reports regarding the effects of DEX on FN biosynthesis yield seemingly discordant experimental outcomes. For example, our study and previous investigations suggest that individual HTM cell strains may react differently to DEX exposure both because of strain-specific receptor-mediated sensitivities to the corticosteroid and possibly different dose-dependent effects. In addition, individual cell strains may express metalloproteases and metalloprotease inhibitors differently in such a way that there are unique modifications of the ECM, either raising or lowering the total amount of FN. The stability of FN messenger RNA may be variable between strains; therefore, even at lower levels of expression, the messenger might be less affected catabolically.

The temporal onset of DEX-generated increases in the FN content of sensitive HTM cells corresponded to the glucocorticoid-mediated alteration of the cell cytoskeleton previously reported by our laboratory. That is, the glucocorticoid-induced formation of cross-linked actin filamentous networks (CLANS) in the perinuclear region of TM cells was seen in the same cell strains that had FN-based sensitivity to DEX. Specifically, up to 60% of cells from strains HTM2, HTM4, and HTM10 form CLANS after 14 d of $10^{-7}$ mol/l DEX treatment; cells from strain HTM6 do not. We currently are evaluating whether DEX-induced alterations of the ECM and cell cytoskeleton precede one another or occur synchronously.

It is not surprising that there is a good correlation between the DEX-induced changes in the microfilaments and FN because these cellular components are linked indirectly through membrane receptors (integrins) and connecting proteins ($\alpha$-actinin, vinculin, and talin). Such a proven linkage of the cell cytoskeleton to the ECM is important because it reinforces the hypothesis that alterations of either the extracellular environment or internal structure of TM cells can affect cell function dramatically. As previously mentioned, glucocorticoids directly can alter the phagocytic activity, cell proliferation and migration, protein secretion, extracellular protease activity, and GAG content of TM tissue or cultured cells. Extrapolating from the effects of corticosteroids on cultured HTM cells, it can be hypothesized that major alterations of the environment and metabolic capabilities of TM cells are fundamental to the etiology of steroid-induced glaucoma. Our study found that one indicator of steroid-induced alteration of the TM may be the differential overexpression of FN.

The accumulation of cellular FN in TM cells may not be linked solely to glucocorticoid treatment, but it also may be associated intimately with primary open-angle glaucoma. For example, FN deposition in elderly eyes was most pronounced in the aqueous outflow channels of the eye, especially the endothelium of Schlemm’s canal. A pronounced distribution of FN was seen in the juxtanacaIicular region of the TM, particularly in glaucomatous eyes. Moreover, in a study of aged-matched trabeculectomy samples from normal and glaucomatous patients, the concentration of FN was enhanced significantly (both in the inner wall of Schlemm’s canal and juxtanaCaIicular TM) in patients with advanced open-angle glaucoma. A selective enhancement was shown in the binding of plasma FN from glaucomatous versus normal patients toward collagen types I, II, and III, but not type IV. Thus, a clear trend toward associating enhanced deposition of FN with some forms of glaucoma has been documented in the literature.

**Table 1.** HTM4 increases in fibronectin of DEX-treated samples over that of controls using different electrophoretic techniques

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<th>% Increase (over control)</th>
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**Key words:** fibronectin, steroid-induced glaucoma, trabecular meshwork, dexamethasone, extracellular matrix

**References**

4. Armaly MF: Corticosteroid glaucoma. In Glaucoma, Vol II,


