Washington University School of Medicine
Digital Commons@Becker

Open Access Publications

5-16-2011

Evaluation of the toxicity of triamcinolone acetonide and dexamethasone sodium phosphate on human lens epithelial cells (HLE B-3)

Ashish Sharma
University of California - Irvine

Ashkan Pirouzmanesh
University of California - Irvine

Jayaprakash Patil
University of California - Irvine

M. F. Estrago-Franco
University of California - Irvine

Leandro Cabral Zacharias
University of California - Irvine

See next page for additional authors

Follow this and additional works at: https://digitalcommons.wustl.edu/open_access_pubs
Please let us know how this document benefits you.

Recommended Citation

Sharma, Ashish; Pirouzmanesh, Ashkan; Patil, Jayaprakash; Estrago-Franco, M. F.; Zacharias, Leandro Cabral; Andley, Usha P.; Kenney, M. Cristina; and Kuppermann, Baruch D., "Evaluation of the toxicity of triamcinolone acetonide and dexamethasone sodium phosphate on human lens epithelial cells (HLE B-3)." Journal of Ocular Pharmacology and Therapeutics. 27, 3. 265-271. (2011).
https://digitalcommons.wustl.edu/open_access_pubs/2800

This Open Access Publication is brought to you for free and open access by Digital Commons@Becker. It has been accepted for inclusion in Open Access Publications by an authorized administrator of Digital Commons@Becker. For more information, please contact vanam@wustl.edu.

Authors
Ashish Sharma, Ashkan Pirouzmanesh, Jayaprakash Patil, M. F. Estrago-Franco, Leandro Cabral Zacharias, Usha P. Andley, M. Cristina Kenney, and Baruch D. Kuppermann

This open access publication is available at Digital Commons@Becker: https://digitalcommons.wustl.edu/open_access_pubs/2800

JOURNAL OF OCULAR PHARMACOLOGY AND THERAPEUTICS
Volume 27, Number 3, 2011
© Mary Ann Liebert, Inc.
DOI: 10.1089/jop.2010.0120

Evaluation of the Toxicity of Triamcinolone Acetonide and Dexamethasone Sodium Phosphate on Human Lens Epithelial Cells (HLE B-3)

Ashish Sharma,[1,2,*] Ashkan Pirouzmanesh,[1,*] Jayaprakash Patil,[1,3]
M. F. Estrago-Franco,[1] Leandro Cabral Zacharias,[1,4] Aslan Pirouzmanesh,[1] Usha P. Andley,[5]
M. Cristina Kenney,[1] and Baruch D. Kuppermann[1]

Abstract

Purpose: The purpose of this study was to compare the *in vitro* effects of triamcinolone acetonide (TA) and dexamethasone sodium phosphate (DEX) on human lens epithelial cells (HLE B-3).
Methods: HLE B-3 cells were exposed for 24 h to commercially available TA (c-TA) and dimethylsulfoxide-solubilized TA (s-TA). The cells were treated with 1,000 (clinical dose), 750, 500, 200, and 100 μg/mL concentrations of c-TA, s-TA, and supernatant for 24 h. The cells were also treated with DEX at 2, 1, 0.5, 0.2, 0.1 (clinical dose), and 0.05 mg/mL. Cell viability, caspase-3/7 activity, and DNA fragmentation analyses were performed.
Results: The mean cell viabilities of HLE B-3 after exposure to c-TA at 1,000, 750, 500, 200, and 100 μg/mL were significantly reduced compared with control untreated cells. The s-TA also significantly reduced cell viability at 1,000, 750, and 500 μg/mL compared with dimethylsulfoxide control. The supernatant did not reduce cell viability. Caspase-3/7 activity significantly increased after treatment with c-TA and s-TA. DNA laddering revealed bands at 200 bp intervals with both c-TA at ≥ 100 μg/mL and s-TA at ≥ 500 μg/mL. The cell viabilities of HLE B-3 after 24 h exposure to DEX were significantly reduced at 2 and 1 mg/mL but not at lower concentrations tested. Caspase-3/7 activities in HLE B-3 cells were not increased significantly after treatment with DEX at any dose tested. DNA laddering did not reveal any band at any dose tested.
Conclusion: This study showed that TA at its clinical dose (1,000 μg/mL) in both commercial preparation and solubilized forms decrease HLE B-3 cell viability through an apoptotic pathway. DEX at its clinical dose (0.1 mg/mL) does not decrease cell viability or cause any increase of caspase-3/7 activity. This study suggests that for long-term sustained-release devices, DEX may be less damaging to human lens cells than TA.

Introduction

THE RECENT FOCUS on the angiostatic and antipermeability properties of steroids has encouraged clinicians to greatly extend the use of intravitreal corticosteroids in a variety of ocular conditions. Triamcinolone acetonide (TA) is an insoluble crystalline steroid that acts as a depot when injected intravitreally. For its prolonged duration of action, TA is broadly used in treatment of a variety of ocular diseases. Intravitreal TA (IVTA) is extensively used in treatment of macular edema due to diabetic retinopathy,[1,2] venous occlusive disease,[3] ocular inflammation,[4,5] and choroidal neovascularization.[6] The anti-inflammatory potency of TA is similar to that of methylprednisolone but significantly less compared with that of dexamethasone.[7] The biological half-life of parenterally administered TA is 18–36 h,[8] whereas the mean elimination half-life in nonvitrectomized eyes is 18.6 days, with measurable concentrations of TA for ~3 months after intravitreal injection in nonvitrectomized eyes.[8]

This study was presented in part at the Annual Meeting of the Association for Research in Vision and Ophthalmology, Ft. Lauderdale, Florida, May 2008.
[1]Gavin Herbert Eye Institute Department of Ophthalmology, School of Medicine, University of California at Irvine, Irvine, California.
[2]Lotus Eye Care Hospital, Coimbatore, India.
[3]Royal Lancaster Infirmary, University Hospitals of Morecambe NHS Trust, Lancaster, United Kingdom.
[4]Department of Ophthalmology, University of San Paulo, San Paulo, Brazil.
[5]Department of Ophthalmology and Visual Sciences, Washington University, St. Louis, Missouri.
*These authors equally contributed as co-first authors.

Dexamethasone sodium phosphate (DEX) is also clinically used to reduce intraocular inflammation; but, because it is only available in a soluble form, its duration of action is much less than that of TA.[9] DEX has a half-life of ∼3 h in the rabbit eye, leading to clearance from the vitreous after ∼3 days.[9] For its short duration of action, intravitreal DEX has enjoyed a limited role in the management of chronic and/or refractory posterior segment diseases. However, advances in new sustained-release devices containing DEX have brought recent excitement in the use of DEX for management of a variety of chronic/refractory ocular conditions such as macular edema.[10]

Studies have shown that although the steroids are injected into the vitreous, there is diffusion into the anterior chamber, and as a result, elevated concentrations of the steroids can be found in that region.[8,11,12] Under these circumstances, the lens epithelial cells would be exposed to high concentrations of steroids, which may have a negative effect because the terminal differentiation of the epithelial cells into lens fibers is essential for development and growth of the lens.[13–16] Therefore, steroid-induced damage to these parental lens cells could disrupt the normal biological processes and contribute to cataract formation.

Adverse events typically associated with corticosteroid therapy include cataract formation and increased intraocular pressure.[17,18,19] The purpose of this study was to compare the *in vitro* effects of 2 steroids that are used clinically, TA and DEX, to determine their cytotoxic effects upon a human lens epithelial cell line (HLE B-3).

Methods

Cell culture

HLE B-3 cells (provided by Usha P. Andley) were grown in tissue culture in minimum essential medium containing Eagle's salts, L-glutamine, gentamicin (50 μg/mL), and 20% fetal bovine serum. The cells were plated in 6- and 24-well plates (Becton Dickinson Labware, Franklin Lakes, NJ) for cell viability (5.5×10^5 cells per well) and caspase-3/7 (1.5×10^5 cells per well) assays, respectively. Passages 15–20 were used for the experiments. As there are variations in enzyme activity with passage and time, experiments were performed in triplicates and all the experiments were repeated 3 times.

Exposure to drug

Commercially available TA (c-TA; Kenalog®; BMS, Princeton, NJ) was centrifuged at 5,000 rpm for 1 min and the supernatant was removed. To generate the solubilized TA (s-TA), the pellet was resuspended in equivalent amounts of dimethylsulfoxide (DMSO) to achieve the same concentration of TA found in the commercial suspension. The cells were treated for 24 h with 1,000 (clinical dose), 750, 500, 200, and 100 μg/mL concentrations of c-TA, s-TA, and the supernatant. Other cells were treated for 24 h with DEX (APP, Schaumburg, IL) at 2,000, 1,000, 500, 200, 100 (clinical dose), and 50 μg/mL concentrations.

Cell viability assay

Cell viability assay was performed as previously described.[20] Briefly, cells harvested from the 6-well plates by treatment with 0.2% trypsin–EDTA were then incubated at 37°C for 5 min. The cells were centrifuged at 1,000 rpm for 1 min and then resuspended in 1 mL of culture medium. Automated cell viability analysis was performed (ViCell analyzer; Beckman Coulter, Inc., Fullerton, CA). The analyzer performed an automated trypan blue dye-exclusion assay, generating percentages of viable cells.

Caspase-3/7 assay

Caspase-3/7 activities were detected with carboxyfluorescein apoptosis detection kits (FLICA; Immunochemistry Technologies LLC, Bloomington, MN). At the designated time period, the wells were rinsed briefly with fresh culture media, replaced with 300 μL/well of 1×FLICA solution in culture media, and incubated at 37°C for 1 h under 5% CO_2. Cells were washed with phosphate-buffered saline. The following controls were included: untreated HLE B-3 cells without FLICA were used as a background control; untreated HLE B-3 cells with FLICA for comparison of caspase activity of treated cells; wells without cells with buffer alone; tissue culture plate wells without cells with culture media + DMSO to exclude cross-reaction of FLICA with DMSO + culture media; and HLE B-3 cells with DMSO and FLICA to account for any cross-fluorescence between untreated cells and DMSO.

Quantitative calculations of caspase activities were performed with a fluorescence image scanning unit instrument (FMBIO III; Hitachi, Yokohama, Japan). The caspase activity was measured as the average signal intensity of the fluorescence of the pixels in a designated spot, that is, mean signal intensity.

DNA fragmentation assay

HLE B-3 cells (5×10^6) were plated overnight in 100-mm dishes and then incubated for another 24 h with c-TA, s-TA, and DEX in serum-free medium. DNA was extracted (QIAamp DNA Micro kit; Qiagen, Hilden, Germany) according to the manufacturer's instructions. Samples were separated by electrophoresis on 3% agarose gels and stained with 5% ethidium bromide. A 100-bp marker was used and images were captured with a fluorescence image scanning instrument (FMBIO III; Hitachi).

Statistical analysis

Data were subjected to statistical analysis by ANOVA (Prism, ver. 3.0; GraphPad Software, Inc., San Diego, CA). Newman–Keuls multiple-comparison test was done to compare the data within each experiment. $P < 0.05$ was considered statistically significant. Error bars in the graphs represent SEM, with experiments performed in triplicate.

Results

Cell viability studies

c-TA preparation. The mean cell viabilities of HLE B-3 after exposure to c-TA at 1,000, 750, 500, 200, and 100 μg/mL were 26.3 ± 5.8 ($P < 0.001$), 34.0 ± 3.2 ($P < 0.001$), 43.6 ± 3.3 ($P < 0.001$), 54.3 ± 11.7 ($P < 0.001$), and 71.6 ± 4.4 ($P < 0.01$), respectively, compared with control untreated cells (89.7 ± 0.5; Fig. 1A).

s-TA preparation. HLE B-3 cells exposed to s-TA also had a significantly reduced mean cell viability of 20.1 ± 2.5 ($P < 0.001$), 30.4 ± 6.6 ($P < 0.001$), and 36.2 ± 2.4 ($P < 0.001$) at

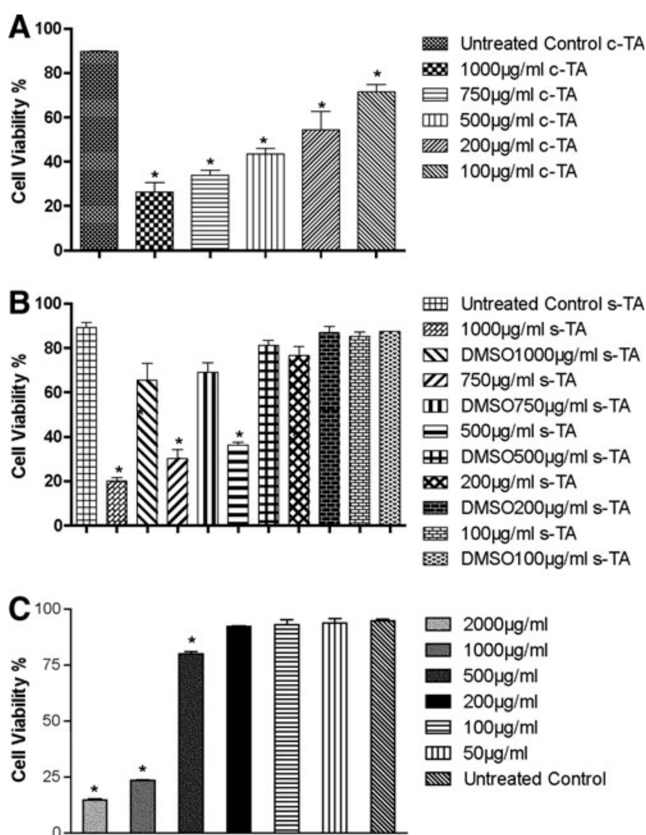

FIG. 1. **(A)** c-TA causes dose-related cell viability loss of HLE B-3 cells, starting at a concentration of 100 µg/mL (*$P < 0.001$). **(B)** s-TA treatment causes dose-related decrease in cell viability of HLE B-3 cells, starting at a concentration of 500 µg/mL (*$P < 0.001$). **(C)** DEX causes dose-related cell viability loss of HLE B-3 cells, beginning at a concentration of 500 µg/mL (*$P < 0.001$). c-TA, commercially available triamcinolone acetonide; DEX, dexamethasone sodium phosphate; HLE, human lens epithelial; s-TA, solubilized TA; DMSO, dimethylsulfoxide.

1,000, 750, and 500 µg/mL, respectively, compared with equivalent DMSO control cultures. At 200 and 100 µg/mL, mean cell viabilities were 76.6 ± 7.2 ($P > 0.05$), and 85.3 ± 3.4 ($P > 0.05$), respectively, compared with equivalent DMSO control cultures. Values of DMSO controls at 1,000, 750, 500, 200, and 100 µg/mL were 65.5 ± 13.0, 69.0 ± 7.4, 81.2 ± 4.0, 86.9 ± 4.7, and 87.6 ± 0.0, respectively. Untreated control culture cell viability was 89.3 ± 3.9 (Fig. 1B).

TA supernatant

The supernatant alone did not reduce the viability of HLE B-3 cells at any concentration. The mean cell viabilities at 1,000, 750, 500, 200, and 100 µg/mL were 85.9 ± 2.7 ($P > 0.05$), 85.3 ± 4.6 ($P > 0.05$), 85.3 ± 4.3 ($P > 0.05$), 84.8 ± 6.4 ($P > 0.05$), and 88.1 ± 3.6 ($P > 0.05$), respectively, compared with control untreated cells (89.4 ± 2.7) (figure not shown).

Dexamethasone sodium phosphate

The mean cell viabilities of HLE B-3 after 24 h exposure to DEX at 2,000, 1,000, 500, 200, 100 (clinical dose), and 50 µg/mL were 14.8 ± 0.8 ($P < 0.001$), 23.6 ± 0.2 ($P < 0.001$), 80.1 ± 1.3 ($P < 0.001$), 92.4 ± 0.3 ($P > 0.05$), 93.1 ± 2.9 ($P > 0.05$), and 93.7 ± 2.9 ($P > 0.05$), respectively, compared with control untreated cells (94.9 ± 0.9) (Fig. 1C).

Caspase-3/7 activity

c-TA preparation. The mean caspase-3/7 activity of HLE B-3 after 24 h exposure to c-TA at 1,000, 750, 500, 200, and 100 µg/mL was 13,407.0 ± 1,079.7 ($P < 0.001$), 13,848.0 ± 1,496.3 ($P < 0.001$), 11,660.0 ± 590.3 ($P < 0.001$), 11,701.3 ± 611.7 ($P < 0.001$), and 10,814.6 ± 947.3 ($P < 0.001$), respectively, compared with control untreated cells (2,600.0 ± 510.9) (Fig. 2A).

s-TA preparation. Caspase-3/7 activity in HLE B-3 significantly increased after treatment with s-TA for 24 h. The cells treated with s-TA 1,000, 750, 500, 200, and 100 µg/mL showed mean fluorescence of 10,160.0 ± 498.2 ($P < 0.001$), 10,886.5 ± 1,429.0 ($P < 0.001$), 8,308.5 ± 485.7 ($P < 0.001$), 7,632.0 ± 914.9 ($P < 0.001$) and 2,932.5 ± 830.8 ($P > 0.05$), respectively. Values for untreated cells and DMSO-equivalent cultures for 1,000, 750, 500, 200, and 100 µg/mL were 3,479.0 ± 281.4 and 3,232.7 ± 24.5, 3,057.7 ± 222.9, 3,325.2 ± 883.6, 3,867.5 ± 116.6, and 3,215.0 ± 487.9, respectively (Fig. 2B).

TA supernatant

Caspase-3/7 activities in HLE B-3 cells were not increased significantly after treatment with supernatant at all concentrations tested. The mean caspase-3/7 activity of HLE B-3 after 24 h exposure to TA supernatant at 1,000, 750, 500, 200, and 100 µg/mL and untreated cells were 3,999.80 ± 454.5 ($P > 0.05$), 3,720.9 ± 374.6 ($P > 0.05$), 3,746.0 ± 502.0 ($P > 0.05$), 4,069.0 ± 239.0 ($P > 0.05$), and 3,910.0 ± 579.8276 ($P > 0.05$), respectively, compared with control untreated cells (3,650.5 ± 637.1) (figure not shown).

Dexamethasone sodium phosphate

Caspase-3/7 activity in HLE B-3 was not significantly increased after treatment with DEX for 24 h. The mean caspase-3/7 activity of HLE B-3 after 24 h exposure to DEX at 2,000, 1,000, 500, 200, 100 (clinical dose), and 50 µg/mL were 3,998.0 ± 457.9 ($P > 0.05$), 4,279.1 ± 521.8 ($P > 0.05$), 3,815.7 ± 714.8 ($P > 0.05$), 4,470.6 ± 1,484.3 ($P > 0.05$), 3,840.5 ± 690.8 ($P > 0.05$), and 4,780.5 ± 649.8 ($P > 0.05$), respectively, compared with control untreated cells (3,983.6 ± 730.2) (Fig. 2C).

DNA fragmentation assay

c-TA preparation. Caspase-3/7 is the hallmark of apoptosis because it is the final common pathway of apoptosis. To verify apoptotic activity, DNA fragmentation analysis was performed, which showed DNA bands laddered in ~200 bp increments, consistent with apoptosis (Fig. 3A).

s-TA preparation. DNA fragmentation analysis showed bands in ~200 bp increments, consistent with apoptosis (Fig. 3B).

Dexamethasone sodium phosphate

DNA laddering did not reveal any band, corroborating the lack of apoptosis evidenced by the absence of caspase-3/7 activity (Fig. 3C).

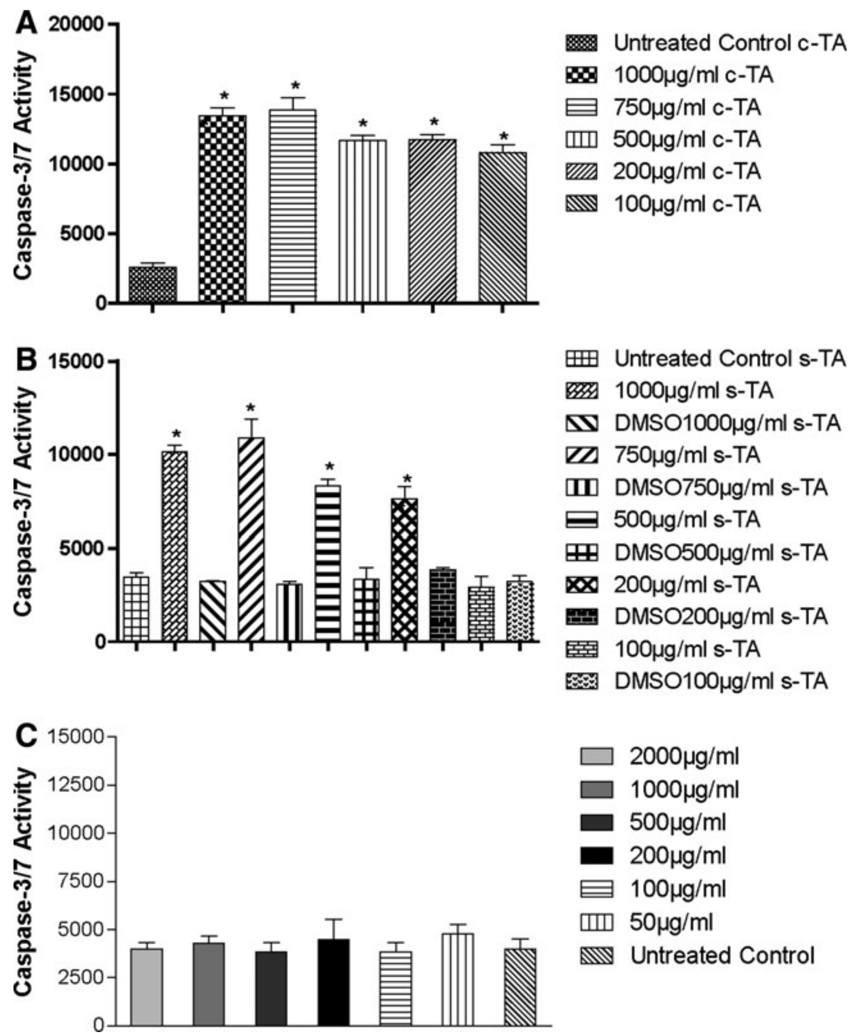

FIG. 2. **(A)** c-TA treatment causes increased caspase-3/7 activity at all concentrations tested (*$P < 0.001$). **(B)** Treatment with s-TA causes higher levels of caspase-3/7 activity at all concentrations tested (*$P < 0.001$) except 100 µg/mL ($P > 0.05$). **(C)** DEX did not show caspase-3/7 activity at any of the concentrations tested ($P > 0.05$).

Discussion

The use of intravitreal corticosteroids in the management of a variety of ocular conditions has recently gained widespread acceptance. Formation and/or progression of cataract are common complications of intravitreal steroids.[19,21,22] In a study by Jonas et al., IVTA in an elderly population of patients led to clinically significant cataract formation and eventual cataract surgery in about 15%–20% of eyes within 1 year.[23] A single IVTA injection was noted to induce posterior subcapsular cataract development, whereas multiple injections result in all-layer cataract progression.[21] Although cataract is a reversible cause of vision loss, with the recent improvements in the cataract surgery techniques, it is now considered to be a relatively low-risk procedure, and it is generally associated with more long-term complications in patients with chronic posterior segment diseases.[24,25] It is therefore of clinical significance to identify whether 1 steroid formulation is less cataractogenic than another.

Loss of cell viability resulting from triggered apoptosis or necrosis in HLE cells has been shown to be involved in the pathogenesis of cataract formation.[26,27] Glucocorticoids induce apoptosis in hematological cells and are used as a chemotherapeutic agent for leukemias, lymphomas, and myeloma. Glucocorticoid-mediated apoptotic cell death could be the final result of the negative modulation of proinflammatory cytokines or glucocorticoid receptor alteration[28,29] or may be a consequence of cell cycle arrest.[30] Charakidas et al. demonstrated the presence of lens epithelial cell apoptosis and proliferation in human age-related cortical cataract.[31] Although they found a relatively low rate of apoptotic death, which they felt was unlikely to cause significant lenticular opacity, it has been recognized that steroids can induce apoptosis,[32] and it is possible that the higher rate of apoptotic death might lead to cataract formation. For example, Li et al. have shown that lens epithelial cell apoptosis occurs in calcimycin-induced cataract formation.[33]

In our study, cell viability assays have demonstrated that both TA and DEX have cytotoxic effects on the HLE B-3 cells in a concentration-dependent fashion, although the effect was more pronounced in cells treated with TA. The usual clinical dosage of IVTA is 4 mg, and assuming that the vitreous volume is 4 mL, the intravitreal clinical concentration of TA would be 1,000 µg/mL (assuming equal dispersion of the drug throughout the vitreous cavity, although typically this does not happen in a nonvitrectomized eye as the drug tends to aggregate). Our study shows that this "clinically equivalent" dose and also doses as low as 1/5 of the clinical dose of both crystalline c-TA and s-TA decrease HLE cell viability. In contrast, the assigned clinical dosage of

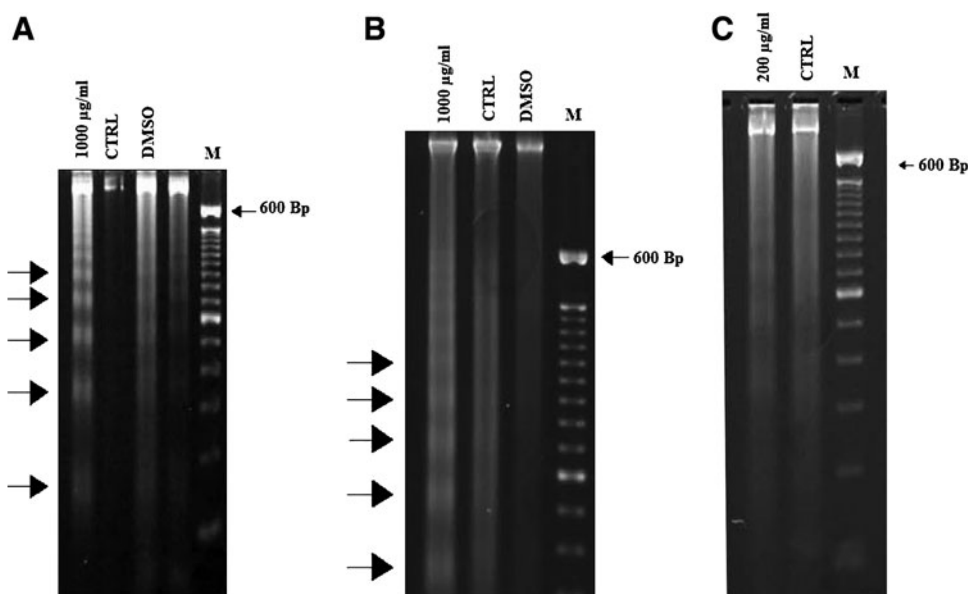

FIG. 3. **(A)** DNA laddering analysis after 24 h exposure to c-TA. DNA bands that laddered in ~200 bp increments are consistent with apoptosis. **(B)** DNA laddering analysis after 24 h exposure to s-TA showed bands that laddered at ~200 bp increments, which are consistent with apoptosis. **(C)** DNA laddering analysis showed that after 24 h exposure to DEX, no bands were observed. M, marker; CTRL, control.

intravitreal dexamethasone is 0.1 mg/mL (100 μg/mL), which is 10 times below the ranges that caused loss of lens cell viability in the HLE B-3 cells. The observed greater toxic potential of TA compared with DEX has been recently shown by Yeung et al. in a cultured retinal pigment epithelium (RPE) cell line.[34] Although many clinical studies have shown cataract development and progression after intravitreal use of TA,[21,35,36] to the best of our knowledge, this is the first study comparing the effect of doses of TA and DEX used clinically on lens epithelial cells *in vitro*.

Loss of cell viability can be attributed to both apoptosis and/or necrosis. Caspase-3/7 is a key effector in the apoptosis pathway, and its activation signals the full commitment to disassembly of the cell. It is known that caspase detects apoptotic and dying cells, but once the cell is dead it will not be caspase positive. Because of high numbers of dead cells at higher doses (1,000 μg/mL), we do not see dose-dependant increase at higher c-TA doses. Our study demonstrates that the loss of cell viability in TA-treated HLE B-3 cells is associated with activation of caspase-3/7 pathway, suggesting an involvement of an apoptotic mechanism, which was then further supported by DNA fragmentation analysis. In contrast, caspase-3/7 activities in HLE B-3 cells were not significantly increased after treatment with DEX at all concentrations tested. Other studies have shown that dexamethasone causes necrosis on corneal endothelial cells,[37] but it does not cause apoptosis in retinal pigment epithelial cells.[38] However, recently, Petersen et al. reported that DEX induces caspase-3 apoptosis in HLE cells. This could be due to difference in cell line used and different conditions of incubation. To mimic normal physiological behavior of HLE cells, we used 20% serum for incubation and treatment, whereas Petersen et al. used serum-free culture media during treatment.[39] Our findings indicate that the cytotoxic effects of TA and DEX are mechanistically different. Further, lower solubility of TA leads to slower, and perhaps unequal, dispersion in vitreous and, therefore, variable concentration at different locations in vitreous cavity. We hypothesize that this could add to cataractogenic toxicity of TA if higher levels remain near the posterior pole of the lens.

We acknowledge that there are limitations to our study. First, concentrations used in this study cannot be directly extrapolated to clinical practice, as this study was performed *in vitro*. In addition, the cells were exposed for only 24 h, which is not the case in clinical conditions, although by looking for subtle downstream changes such as mitochondrial membrane potential changes and caspase upregulation we are able to detect early effects that are likely to be predictive of later damage. Third, TA drug crystals came in direct contact with the cells, which happens in clinical conditions such as in vitrectomized eyes. However, to eliminate the crystal contact effect, the TA was solubilized and there was a decrease in cell viability and an increase in caspase-3/7 activity. In general, the *in vitro* cell toxicity experiments have been predictive or corroborative of clinically observed toxicity with other agents such as vital dyes.[20] Regardless, the results of this study suggest that doses of TA clinically used may be more cytotoxic to HLE cells than DEX and, hence, perhaps, more cataractogenic. Despite having more potency, DEX has also been shown to be less cytotoxic in other ocular cell lines than TA.[34,40–44] The varying levels of cytotoxicity for DEX and TA may be due to differences in their chemical formulations. TA has been shown to be more toxic than DEX primarily because of its crystalline formulation. Although these crystals make TA an excellent choice for long-term drug delivery, on the other hand, these crystals have been shown as a cause of toxicity. Szurman et al. showed that filter-purified, nonadherent TA is nontoxic to ARPE-19 cells at concentrations of up to 1,000 μg/mL.[44] However, filtering this compound may result in decreased efficacy of TA, making it undesirable to use clinically. To summarize, the decreased lens epithelial cell toxicity observed *in vitro* with DEX compared with TA, combined with the greater potency of DEX over TA, suggests that DEX may be a better option for prolonged clinical use in the eye.

Acknowledgments

This study was supported by the Discovery Eye Foundation, the Iris and B. Gerald Cantor Foundation, Research to

Prevent Blindness Foundation, The Ko Family Foundation, and Gilbert Foundation, Irvine, CA.

Author Disclosure Statement

All authors declare that no competing financial interests exist.

References

1. Jonas, J.B., and Söfker, A. Intraocular injection of crystalline cortisone as adjunctive treatment of diabetic macular edema. *Am. J. Ophthalmol.* 132:425–427, 2001.
2. Jonas, J.B., Kreissig, I., and Söfker, A. Degenring, R.F. Intravitreal injection of triamcinolone for diffuse diabetic macular edema. *Arch. Ophthalmol*. 121:57–61, 2003.
3. Lee, H., and Shah, G.K. Intravitreal injection of triamcinolone as primary treatment of cystoid macular edema secondary to branch retinal vein occlusion. *Retina* 25:551–555, 2005.
4. Antcliff, R.J., Spalton, D.J., Stanford, M.R., et al. Intravitreal triamcinolone for uveitic cystoid macular edema: an optical coherence tomography study. *Ophthalmology* 108:765–772, 2001.
5. Young, S., Larkin, G., Branley, M., and Lightman, S. Safety and efficacy of intravitreal triamcinolone for cystoid macular oedema in uveitis. *Clin. Exp. Ophthalmol*. 29:2–6, 2001.
6. Challa, J.K., Gillies, M.C., Penfold, P.L., et al. Exudative macular degeneration and intravitreal triamcinolone: 18 month follow up. *Aust. N. Z. J. Ophthalmol*. 26:277–281, 1998.
7. Gilman A.G. *The Pharmacologic Basis of Therapeutics*, 10th ed. New York: McGraw-Hill; 2001.
8. Beer, P.M., Bakri, S.J., Singh, R.J., et al. Intraocular concentration and pharmacokinetics of triamcinolone acetonide after a single intravitreal injection. *Ophthalmology* 110:681–686, 2003.
9. Kwak, H.W., and D'Amico, D.J. Evaluation of the retinal toxicity and pharmacokinetics of dexamethasone after intravitreal injection. *Arch. Ophthalmol.* 110:259–266, 1992.
10. Kuppermann, B.D., Blumenkranz, M.S., Haller, J.A., et al. Dexamethasone DDS Phase II Study Group. Randomized controlled study of an intravitreous dexamethasone drug delivery system in patients with persistent macular edema. *Arch. Ophthalmol.* 125:309–317, 2007.
11. Cheng, L., Banker, A.S., Martin, M., Kozak, I., and Freeman, W.R. Triamcinolone acetonide concentration of aqueous humor after decanted 20-mg intravitreal injection. *Ophthalmology* 116:1356–1359, 2009.
12. Jonas, J.B. Intraocular availability of triamcinolone acetonide after intravitreal injection. *Am. J. Ophthalmol*. 137:560–562, 2004.
13. Piatigorsky, J. Lens differentiation in vertebrates. A review of cellular and molecular features. *Differentiation* 19:134–153, 1981.
14. Coulombre, J.L., and Coulombre, A.J. Lens development: Fibre elongation and lens orientation. *Science* 142:1489–1490, 1963.
15. McAvoy, J.W., Chamberlain, C.G., de Iongh, R.U., Hales, A.M., and Lovicu, F.J. Lens development. *Eye* 13:425–437, 1999.
16. Grainger, R.M., Henry, J.J., Saha, M.S., and Servetnick, M. Recent progress on the mechanisms of embryonic lens formation. *Eye* 6:117–122, 1992.
17. Armaly, M.F. Statistical attributes of the steroid hypertensive response in the clinically normal eye. I. The demonstration of three levels of response. *Invest. Ophthalmol*. 4:187–197, 1965.
18. Becker, B. Intraocular pressure response to topical corticosteroids. *Invest. Ophthalmol.* 4:198–205, 1965.
19. Butcher, J.M., Austin, M., McGalliard, J., and Bourke, R.D. Bilateral cataracts and glaucoma induced by long term use of steroid eye drops. *Br. Med. J.* 309:43, 1994.
20. Narayanan, R., Kenney, M.C., Kamjoo, S., et al. Trypan blue: effect on retinal pigment epithelial and neurosensory retinal cells. *Invest. Ophthalmol. Vis. Sci.* 46:304–309, 2005.
21. Cekiç, O., Chang, S., and Tseng, J.J. Cataract progression after intravitreal triamcinolone injection. *Am. J. Ophthalmol.* 139:993–998, 2005.
22. Ozkiriş, A., and Erkiliç, K. Complications of intravitreal injection of triamcinolone acetonide. *Can. J. Ophthalmol.* 40:63–68, 2005.
23. Jonas, J.B., Kreissig, I., and Degenring, R.F. Cataract surgery after intravitreal injection of triamcinolone acetonide. *Eye* 18:361–364, 2004.
24. Kim, S.J., Equi, R., and Bressler, N.M. Analysis of macular edema after cataract surgery in patients with diabetes using optical coherence tomography. *Ophthalmology* 114:881–889, 2007.
25. Wang, J.J., Klein, R., Smith, W., et al. Cataract surgery and the 5-year incidence of late-stage age-related maculopathy: pooled findings from the Beaver Dam and Blue Mountains eye studies. *Ophthalmology* 110:1960–1967, 2003.
26. Jacob, T.J., Karim, A.K., and Thompson, G.M. The effects of steroids on the human lens epithelium. *Eye* 1 (Pt 6):722–727, 1987.
27. Karim, A.K., Jacob, T.J., and Thompson, G.M. The human lens epithelium; morphological and ultrastructural changes associated with steroid therapy. *Exp. Eye Res.* 48:215–224, 1989.
28. James, E.R., Fresco, V.M., and Robertson, L.L. Glucocorticoid-induced changes in the global gene expression of lens epithelial cells. *J. Ocul. Pharmacol. Ther.* 21:11–27, 2005.
29. Wenk, E.J., Hernandez, M.R., Weinstein, B.I., et al. Glucocorticoid receptor binding in bovine lens. *Invest. Ophthalmol. Vis. Sci.* 22:599–605, 1982.
30. Greenstein, S., Ghias, K., Krett, N.L., and Rosen, S.T. Mechanisms of glucocorticoid-mediated apoptosis in hematological malignancies. *Clin. Cancer Res*. 8:1681–1694, 2002.
31. Charakidas, A., Kalogeraki, A., and Tsilimbaris, M. Lens epithelial apoptosis and cell proliferation in human age-related cortical cataract. *Eur. J. Ophthalmol*. 15:213–220, 2005.
32. Bourcier, T., Forgez, P., and Borderie, V. Regulation of human corneal epithelial cell proliferation and apoptosis by dexamethasone. *Invest. Ophthalmol. Vis. Sci*. 41:4133–4141, 2000.
33. Li, W.C., Kuszak, J.R., Wang, G.M., Wu, Z.Q., and Spector, A. Calcimycin-induced lens epithelial cell apoptosis contributes to cataract formation. *Exp. Eye Res*. 61:91–98, 1995.
34. Yeung, C.K., Chan, K.P., Chan, C.K., Pang, C.P., and Lam, D.S. Cytotoxicity of triamcinolone on cultured human retinal pigment epithelial cells: comparison with dexamethasone and hydrocortisone. *Jpn. J. Ophthalmol*. 48:236–242, 2004.
35. Gillies, M.C., Kuzniarz, M., Craig, J., et al. Intravitreal triamcinolone-induced elevated intraocular pressure is associated with the development of posterior subcapsular cataract *Ophthalmology* 112:139–143, 2005.
36. Gillies, M.C., Simpson, J.M., and Billson, F.A. Safety of an intravitreal injection of triamcinolone: results from a randomized clinical trial. *Arch. Ophthalmol*. 122:336–340, 2004.
37. Chen, W.L., Lin, C.T., Yao, C.C., et al. *In-vitro* effects of dexamethasone on cellular proliferation, apoptosis, and

Na+-K+-ATPase activity of bovine corneal endothelial cells. *Ocul. Immunol. Inflamm*. 14:215–223, 2006.

38. Sibayan, S.A., Kobuch, K., Spiegel, D., et al. Epinephrine, but not dexamethasone, induces apoptosis in retinal pigment epithelium cells *in vitro*: possible implications on the pathogenesis of central serous chorioretinopathy. *Graefes Arch. Clin. Exp. Ophthalmol*. 238:515–519, 2000.
39. Petersen, A., Carlsson, T., Karlsson, J.O., Jonhede, S., and Zetterberg, M. Effects of dexamethasone on human lens epithelial cells in culture. *Mol. Vis.* 14:1344–1352, 2008.
40. Narayanan, R., Mungcal, J.K., Kenney, M.C., Seigel, G.M., and Kuppermann, B.D. Toxicity of triamcinolone acetonide on retinal neurosensory and pigment epithelial cells. *Invest. Ophthalmol. Vis. Sci*. 47:722–728, 2006.
41. Shaikh, S., Ho, S., Engelmann, L.A., and Klemann, S.W. Cell viability effects of triamcinolone acetonide and preservative vehicle formulations. *Br. J. Ophthalmol.* 90:233–236, 2006.
42. Chung, H., Hwang, J.J., Koh, J.Y., Kim, J.G., and Yoon, Y.H. Triamcinolone acetonide-mediated oxidative injury in retinal cell culture: comparison with dexamethasone. *Invest. Ophthalmol. Vis. Sci.* 48:5742–5749, 2007.
43. Chang, Y.S., Wu, C.L., Tseng, S.H., Kuo, P.Y., and Tseng, S.Y. Cytotoxicity of triamcinolone acetonide on human retinal pigment epithelial cells. *Invest. Ophthalmol. Vis. Sci*. 48:2792–2798, 2007.
44. Szurman, P., Kaczmarek, R., and Spitzer, M.S. Differential toxic effect of dissolved triamcinolone and its crystalline deposits on cultured human retinal pigment epithelium (ARPE19) cells. *Exp. Eye Res.* 83:584–592, 2006.

Received: August 29, 2010
Accepted: March 28, 2011

Address correspondence to:
Dr. Baruch D. Kuppermann
Gavin Herbert Eye Institute
Department of Ophthalmology
University of California at Irvine
118 MedSurge I
Irvine, CA 92697

E-mail: bdkupper@uci.edu