J.Natn.Sci.Foundation Sri Lanka 2021 49 (2): 295 - 302
DOI: http://dx.doi.org/10.4038/jnsfsr.v49i2.9636

RESEARCH ARTICLE

Dexamethasone induced glaucoma associated features exhibited differentially in retinal ganglion cells and human trabecular meshwork cells

N Maurya and I Ghosh*
School of Environmental Sciences, Jawaharlal Nehru University, New Delhi, India.

Submitted: 25 November 2019; Revised: 12 November 2020; Accepted:26 March 2021

Abstract: Several studies have reported long-term dexamethasone application to cause glaucoma, with understanding of the underlying mechanism being incomplete. As Trabecular Meshwork (TM) cells and Retinal Ganglion Cells (RGCs) are primary targets for glaucomatous induction, studying the effect of dexamethasone on these cells becomes significant. The present work used an in vitro approach where Human Trabecular Meshwork (HTM) and RGC-5 cell lines were subjected to dexamethasone treatment and analysed. This study reports differential response of both types of cells towards same dexamethasone challenge, where HTM cells exhibited altered morphology, decreased cell survival, elevated reactive oxygen species (ROS) status along with increased expression of pro-apoptotic markers (p53, Bax) while RGC-5 cells did not show signs of stress prima facie. Since dexamethasone has been reported to increase intra-ocular pressure (IOP) in vivo thereby depleting nutrient supply to retinal neuronal cells, treatment of RGC-5 cells with serum free medium was performed. In this condition, RGC-5 cells exhibited glaucoma-associated stress features. Elevated expression of myocilin in both dexamethasone treated Human Trabecular Meshwork (HTM) cells and RGC-5 cells was observed. A significant upregulation of myocilin and downregulation of γ-synuclein were also observed in serum free medium treated RGC-5 cells. In conclusion, RGCs do not get affected directly by dexamethasone due to rich nutrient supply unlike HTM cells that are affected, as they exist in regions of less nutrient availability in vivo. Dexamethasone affects RGCs indirectly by depleting their nutrients. Thus, maintenance of rich nutrient supply to RGCs that are under dexamethasone administration may help prevent its damaging effect.

Keywords: Dexamethasone, human trabecular meshwork cells, in vitro, oxidative stress, retinal ganglion cells.

INTRODUCTION

Dexamethasone is a synthetic steroidal 9-fluoro glucocorticoid which has immunosuppressive and anti-inflammatory properties, that has been listed among the most important medicines needed in basic health system in the 18th WHO Model List of Essential Medicines (World Health Organization, 2013). It is an anti-emetic and anti-allergic compound used in health conditions ranging from many minor ailments to serious ones. Dexamethasone's effect depends on the type of cellular system in which it is administered (Gruver-Yates & Cidlowski, 2013), along with the time for which the cellular system is exposed. Studies suggest the involvement of a glucocorticoid (GC), which is responsible for both anti- and pro-apoptotic effects of dexamethasone in different cellular systems (Gruver-Yates & Cidlowski, 2013). In addition to its medicinal benefits, a large volume of medical literature suggests its involvement in induction of many side effects on the basis of available documentation and incidence of adverse effects of its related corticosteroids. Its adverse side effects range from acne, weight gain and increased risk of infections, to more severe conditions like cataract, peptic ulcers, hypertension, type 2 diabetes mellitus, osteoporosis and glaucoma (*https://reference.*

* Corresponding author (iloraghosh17@gmail.com; https://orcid.org/0000-0003-3957-8152)

This article is published under the Creative Commons CC-BY-ND License (http://creativecommons.org/licenses/by-nd/4.0/). This license permits use, distribution and reproduction, commercial and non-commercial, provided that the original work is properly cited and is not changed in anyway.

medscape.com/drug/decadron-dexamethasone-intensol-dexamethasone-342741).

According to many studies, dexamethasone induced apoptosis is reactive oxygen species (ROS) related, as by the administration of superoxide dismutase (SOD), the apoptotic induction get attenuated (Oshima *et al*., 2004). Studies indicate that dexamethasone is involved in causing ocular hypertension (OHT) in susceptible individuals if its use is sustained, and in long term use, it may cause vision loss (Goldmann, 1962). This OHT induced by corticosteroids mimics primary open angle glaucoma (POAG) aspects (Bernstein & Schwartz, 1962). Being a glucocorticosteroid, dexamethasone increases intraocular pressure (IOP), reduces conventional aqueous outflow and induces myocilin expression in the Trabecular Meshwork (TM) tissue in mice model of GC induced OHT (Patel *et al*., 2017). It is reported to activate transforming growth factor-β (TGFβ) signalling that is responsible for endoplasmic reticulum (ER) stress, extracellular matrix (ECM) accumulation and IOP elevation (Kasetti *et al*., 2018). An early study showed that the treatment of TM cell cultures with dexamethasone for 24 hours elevated the expression of myocilin / TM-induced glucocorticoid response protein (TIGR) mRNA and the effect was more magnified after 72 hours of treatment (Tamm *et al*., 1999). There is accumulation of myocilin in the cytoplasm of such cells which could be possibly interfering with TM cells' normal functions (Tamm *et al*., 2002). Another instance of dexamethasone affecting Human Trabecular Meshwork (HTM) cells is induction of alteration in DNA methylation status in these cells and this epigenetic modification may affect TM gene expression profile (Matsuda *et al*., 2015). Its role in ROS generation has also been reported in different cellular systems (You *et al*., 2009; Liu *et al*., 2018). Thus, overall it may cause or aggravate the glaucomatous condition in a number of ways. Hence, there are some reported ways in which dexamethasone is known to affect HTM cells leading to pathological changes in it, but complete understanding of how this corticosteroid works to inflict and cause disease is not understood. The exact molecular mechanism of glucocorticoid induced glaucoma is elusive (Zhuo *et al*., 2010).

Retinal Ganglion Cells (RGCs) are also affected by dexamethasone exposure but there are fewer evidences to support it as compared to those for HTM cells. Also, there have been certain reports which show that RGCs are actually protected by dexamethasone under certain stressful conditions like hyperglycemia (Pereiro *et al*., 2018). Ge and group had reported that purified RGCs from rats without additional neurotrophic factors become apoptotic in vitro within 24 hours. of dexamethasone exposure while this situation does not arise in co-cultured RGCs (Ge *et al*., 1999). Also, there has been a recent report which says that there is loss of RGCs in mice due to increased IOP upon dexamethasone exposure but in case of rats, this does not happen as there is a paradoxical decrease in IOP upon dexamethasone treatment (Sato *et al*., 2016). Hence, the effect of dexamethasone on RGCs is actually quite complex and it has not been elucidated completely. The importance of understanding the effect of dexamethasone on both RGCs and HTM cells is therefore quite evident, to know how it induces glaucomatous implications in vivo. The present work thus aimed at elucidating the effects of prolonged exposure of dexamethasone on two different cell lines, i.e. HTM and RGC-5 in relation to glaucomatous pathogenesis.

METHODOLOGY

Low glucose Dulbecco's modified Eagle's medium (LG-DMEM) and antibiotics, namely, gentamycin, penicillin-streptomycin, fungizone and DAPI (4′,6-diamidino-2-phenylindole) were bought from Sigma-Aldrich, USA while the fetal bovine serum (FBS) was obtained from Invitrogen (Gibco), USA. Primary and secondary antibodies were obtained from Santa Cruz Biotechnology Inc., (USA), Sigma Aldrich Chemicals Pvt. Ltd. (USA), Abcam Inc. (USA) and Cell Signaling Tech. (USA). Alexa Fluor 488 and 546 and 2',7'-dichlorodihydrofluorescein diacetate (H_2DCFDA) were from Molecular Probes Inc. (Eugene, OR). MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was bought from Sisco Research Laboratories Pvt. Ltd., India. Dexamethasone was obtained from Sigma-Aldrich, India while all stains were from Spectrachem Pvt. Ltd. All chemicals used were of standard molecular grade.

The HTM cell line is the cellular model for the TM tissue, obtained from human source while the RGC-5 is the immortalized retinal ganglion cell line that is considered to be a useful cellular model to study the mechanisms of cell death and cyto-protection related to glaucoma pathogenesis (Sayyad *et al*., 2017). Further, it expresses the same markers of retinal ganglion cells and neuronal cells as the RGC-precursor-like cell line known as 661W cell line (Sayyad *et al*., 2017).

Cell culture and treatment

Both HTM and RGC-5cells were cultured in LG-DMEM containing 10 % FBS with 100 µl/mL penicillin, 100 g/mL streptomycin, 50 µg/mL gentamycin and 50µg/mL fungizone. RGC-5 cells were sub-cultured

and treated with dexamethasone (2×10^{-7} M for 10 days) after ~70–80 % growth in 10 % FBS-LG-DMEM only because these cells are sufficiently supplied with blood in the in vivo condition. HTM cells on the other hand, were sub-cultured and treated in 1 % FBS- LG-DMEM. This method was adopted to minimize the effect of serum which is absent inaqueous humor where the HTM cells are found. Serum-free media (SFM) containing absolutely no FBS, was also used for treating RGC-5 cells. Dexamethasone stock was prepared in DMSO and it was administered everyday with medium change. The concentration and time of incubation were determined using MTT and ROS assays using hit and trial method (data not shown).

Cell viability assay

MTT assay was done to assess the cell viability. Cells (HTM and RGC-5) seeded in a 24 well plate (10,000 cells per well) were allowed to stick to the wells overnight and treated with dexamethasone at the rate of 2×10^{-7} M continuously for 10 days, with treatment-medium changed every 24 hrs. After completion of treatment period, the medium was sucked out from each well and cells were washed twice with autoclaved PBS (1×, pH 7.2). Then, 5 mg/mL MTT stock (10 µL of stock per well in LG-DMEM) was added and incubated in dark at 37 °C for 2 h. Dimethylsulfoxide (DMSO) (200 µL) was added to each well to solubilize the formazan crystals. Absorbance was measured using Spectramax M5 Spectrophotometer equipped with Soft-Max-Pro 5.4.1 software at 570 nm. Cell viability was calculated in percentage against control samples.

Reactive oxygen species (ROS) assay using H_2DCFDA

ROS production was detected by fluorescence of H_2DCFDA (10 µM) incubated with control and treated sets of cells. After treatment of 70–80 % confluent cells (HTM and RGC-5) grown in 6 well plates with dexamethasone, they were incubated with 10 µM H_2DCFDA solution in dark for 30 min. Then after medium removal, and one wash with autoclaved PBS (1×, pH 7.2), they were scraped out in 200 µL of PBS, collected in eppendorffs and kept in ice. Fluorescence readings were taken in Spectramax M5 Spectrophotometer equipped with SoftMaxPro 5.4.1 software at an excitation wavelength of 488 nm and emission at 530 nm.

Hematoxylin-Eosin staining

Treated and control cells fixed in chilled methanol were stained with hematoxylin- eosin for analyzing morphological changes. For this, methanol was removed and two changes of 99% ethanol for 2 min each were given followed by two changes of 95 % ethanol for 2 min each. After washing with double distilled water for one minute, hematoxylin was spread on the coverslips and kept for 1 min. After thorough washing in running water for 3 min, eosin was applied and kept for 30 s only. Coverslips were rinsed in running tap water for 30 s. Dehydration was done by giving two changes of 95% ethanol for 2 min and again two changes of 99 % ethanol for two minutes each. Mounting was done in DPX and slides were observed under Carl Zeiss (Germany) Microscope equipped with Axiocam camera system having ZenPro software for light microscopy.

Protein estimation by Bradford's method

Protein content of the harvested and radioimmunoprecipitation assay (RIPA) buffer lysed cells was estimated using Bradford (Bradford, 1976) method with BSA as the standard. Absorbance readings were taken at 595 nm in a UV-visible double beam spectrophotometer.

Immuno-cytochemical assay

Changes in the degree of expression and sub-cellular localization of proteins in normal and treated cells grown on coverslips in 12 well plates, was done by immuno-cytochemical analysis. Cells were fixed on coverslips with paraformaldehyde (2 %) for 3 min and excess was quenched with glycine (0.1 M) and permeabilized with Triton X-100 (0.1 %). After blocking with 3 % BSA in PBS, pH 7.2, for 1 h at 37 ºC the cells were incubated with specific primary antibody for 1 h at 37 °C and subsequently washed with autoclaved 1xPBS, pH 7.2 (3 × 10 min). Then, the cells were incubated with fluorescence tagged secondary antibodies solutions in dark for 45 min at 37 °C in humid chamber followed by washing (6 × 10 min) with autoclaved 1×PBS, pH 7.2. Thereafter, the cells were stained with DAPI, washed and mounted in 50 % glycerol in PBS. Fluorescence images were taken using Axioscope microscope (Carl Zeiss, Germany) equipped with epifluorescence and Axiocam camera system coupled with Axio Vision software (Carl Zeiss, Germany). Images were further analysed using freely available ImageJ software.

Enzyme-linked immunosorbent assay (ELISA)

Samples were coated in 96 well plate ensuring that equal amount of protein is loaded per well (1 µg). Blocking with 3 % bovine serum albumin, was followed by incubation with primary antibodies (1:1000) of either diluted in 1.5 % bovine serum albumin (BSA) in phosphate-

buffered saline (PBS) separately. After incubation at 37 °C for 90 min, the samples were removed, the wells were washed 3 times with wash solution and a secondary antibody which is directly conjugated to horse radish peroxidase was added. Signal generated was read in Soft Max Pro software with Tetramethyl benzidine (TMB)-hydrogen peroxide solution prepared freshly.

Immunoblotting

Total protein samples of equal amounts (100µg/lane) were resolved on a 12.5 % SDS-PAGE. Electro-blotting on a PVDF membrane was done at 150 mA for 3 h. The protein profile was checked with amido-black, alternatively washed with PBST and methanol. Blocking of unbound sites on the blot was done using 5 % skimmed milk in PBS, at pH 7.2, for 4 h at room temperature. After 3 times washing with PBST, it was probed with appropriate (anti-Bax and anti-p53) primary antibody and then with either an alkaline phosphatase or horse radish peroxidase conjugated secondary antibody raised against mouse or rabbit, respectively. Blots were then developed either using NBT and BCIP colorimetric system or electrochemical luminescence (ECL). The bands obtained were analysed using freely available ImageJ software.

Statistical analysis

Student's t-test was done to determine the significance of results obtained.

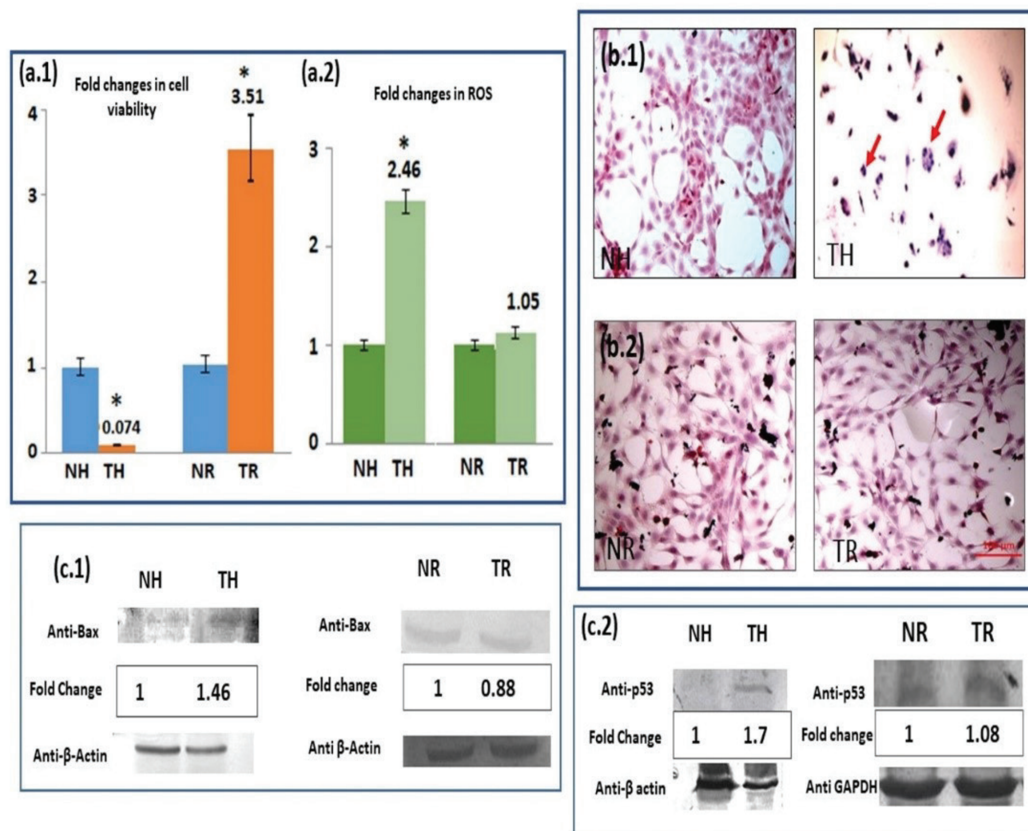

Figure 1: Effect of dexamethasone on HTM and RGC-5 cells
a.1 - Cell viability (MTT assay) for HTM and RGC-5 cells; a.2 - ROS status (H_2DCFDA assay) of HTM cells and RGC-5 cells; b.1 - HE staining of HTM cells; b.2 – HE staining of RGC-5 cells; c.1 - pro-apoptotic marker Bax of HTM cells and RGC-5 cells; c.2 - p53 of HTM cells and RGC-5 cells.
NH: normal untreated HTM cells; TH: dexamethasone treated HTM cells; NR: normal untreated RGC-5 cells; TR: dexamethasone treated RGC-5 cells
* represents significant change at $p < 0.05$). Scale bar represent 100 µm.

RESULTS AND DISCUSSION

Effect of dexamethasone on HTM and RGC-5 cells

The MTT assay results for HTM and RGC-5 cells showed opposite effect upon induction by dexamethasone (2×10^{-7} M for 10 days) (Figure 1.a.1). HTM cells showed a significant ($p < 0.05$) decrease of 0.074 fold while RGC-5cells exhibited a significant ($p < 0.05$) increase in survival of nearly 3.5 fold compared to control set.

The ROS status of the HTM cells was raised (~2.5 fold increase) significantly ($p < 0.05$) while that of RGC-5 cells remained practically unaffected (Figure 1.a.2) by dexamethasone at the specified concentration and time of exposure.

HE staining showed altered HTM cell morphology post dexamethasone treatment with cells becoming irregularly shaped, forming clusters and having shrunken nuclei and cytoplasm. The RGC-5 cells apparently remained unaffected with no signs of stress after dexamethasone exposure at the specific concentration and time of incubation (Figure 1.b.1 and 1.b.2).

Upregulation of Bax (Figure 1.c.1) and p53 (Figure 1.c.2) in case of HTM cells pointed towards apoptotic induction in them while RGC-5 cells remained unaffected. In fact, there was a reduction in expression of Bax (~12 %) in RGC-5 cells treated with dexamethasone, as evident from the immunoblot-analysis.

Effect of serum free medium (SFM) on RGC-5 cells

Upon 4-days long treatment of RGC-5 cells with SFM (time of exposure determined on the basis of MTT and ROS assay results using hit and trial method which is not shown in this article), visible apoptotic features occurred, which were similar to those of HTM cells exposed to dexamethasone. The serum depleted RGC-5 cells exhibited a significant 0.67 fold decrease ($p < 0.05$) in cell survival (Figure 2.a), 2.56 times elevated ROS status (Figure 2.a) and altered morphology (Figure 2.b.1 and 2.b.2). These cells also underwent shrinkage of nuclei and cytoplasm, clustering and structural irregularities similar to dexamethasone exposed HTM cells.

The expression level of p53 in RGC-5 cells did not show significant alteration in SFM treated condition (1.22 fold increase, $p > 0.05$) but its localization changed, from being diffused in the whole cell body in the control set to getting more concentrated in the nuclear region in treated set (Figure 2.c.1). Further, a significant ($p < 0.01$) ~2.1 fold increase in expression of Bax along with its localization in the nuclei of the SFM treated RGC-5 cells is an important apoptotic feature (Figure 2.c.2 and 2.c.3). A significant increase ($p < 0.05$) in expression level of myocilin was observed in case of both HTM (2.054 fold) and RGC-5 cells (3.061 fold) treated with dexamethasone, as revealed by ELISA done for cellular lysates. A 1.413 fold increase in SFM treated RGC-5 cells was also observed ($p < 0.1$) in comparison to the control set (Figure 2.d).

Expression of γ-synuclein showed a decrease (0.79 fold) in RGC-5 cells under SFM treatment in comparison to the control set of cells, as evident from the immunoblotting results (Figure 2.e).

Apoptotic loss of HTM and RGCs is a major factor responsible for glaucoma pathogenesis. It becomes quite clear from the observations that dexamethasone induces apoptotic loss of HTM cells in a p53 dependent manner. An increase in the expression of apoptotic marker Bax is also suggestive of such apoptotic induction. Other characteristics such as increase in ROS and decrease in MTT reduction capacity also support this notion. The initial observations in case of RGC-5 cells treated with dexamethasone such as; no significant changes in cellular morphology, ROS status and expression of p53, Bax along with increase in cell survival, appear to indicate that RGCs do not get stressed under such treatment, but increased expression of myocilin is definitely indicative of glaucoma induction. It is possible that such difference in response of these two types of ocular cells is due to the availability of nutrients. As mentioned above, HTM cells were maintained and treated in 1 % FBS containing medium while RGC-5 cells were maintained and treated in 10% FBS containing medium in order to simulate the actual in vivo condition as closely as possible. HTM tissue is devoid of blood supply and is bathed in aqueous humor but RGCs have access to rich nutrient supply through blood. Thus, it is possible that abundant nutrient supply is responsible for the protection of RGCs against apoptotic loss of these cells in vivo. However, RGCs are not completely spared from detrimental effects of dexamethasone as a significant ~3 fold elevation in expression of myocilin was observed in these cells upon dexamethasone treatment in 10 % FBS containing medium.

It has been reported in many previous studies that dexamethasone is responsible for increasing the IOP upon prolonged exposure in vivo (Chin *et al*., 2017; Patel *et al*., 2017). Increased IOP leads to reduction in

blood supply to the retinal neurons (Findl *et al*., 1997; Alagoz *et al*., 2008). Thus, in an indirect way, prolonged dexamethasone depletes the RGCs of their nutrients, which is a source of stress for these cells. Considering this effect of dexamethasone, simulation of such a nutrient deficient condition in vitro required us to grow RGC-5 cells in SFM. In this condition, visible signs of stress were observed in the RGC-5 cells which were similar to those observed in HTM cells under dexamethasone treatment. Another very strong evidence of induction of glaucoma-associated features was a significant increase in myocilin expression (Resch *et al*., 2008) and a decrease in γ-synuclein expression. Since a reduction in γ-synuclein expression has been reported in a decreased viability of the immortalized RGC-5 cells (Surgucheva *et al*., 2008), a similar observation of reduced γ-synuclein expression in RGC- 5 cells treated with SFM indicates a decrease in their viability and induction of stress. Thus, it can be concluded that dexamethasone exposure to these ocular cells for a prolonged period of time affects HTM cells directly by inducing p53 dependent apoptosis, with upregulation of pro-apoptotic marker Bax and a very important glaucoma associated protein marker myocilin along with other associated features such as reduced cell survival, elevated ROS status and altered morphology. RGC-5 cells on the other hand are indirectly affected by dexamethasone where cellular morphology, and pro-apoptotic markers such as Bax and p53 are not altered, but myocilin is upregulated which is a significant glaucoma associated feature. Another important indirect effect of dexamethasone on RGC-5 cells can be due to nutrient depletion occurring due to raised IOP in vivo. Upon simulation of this condition, RGC-5 cells subjected to SFM showed apoptotic features

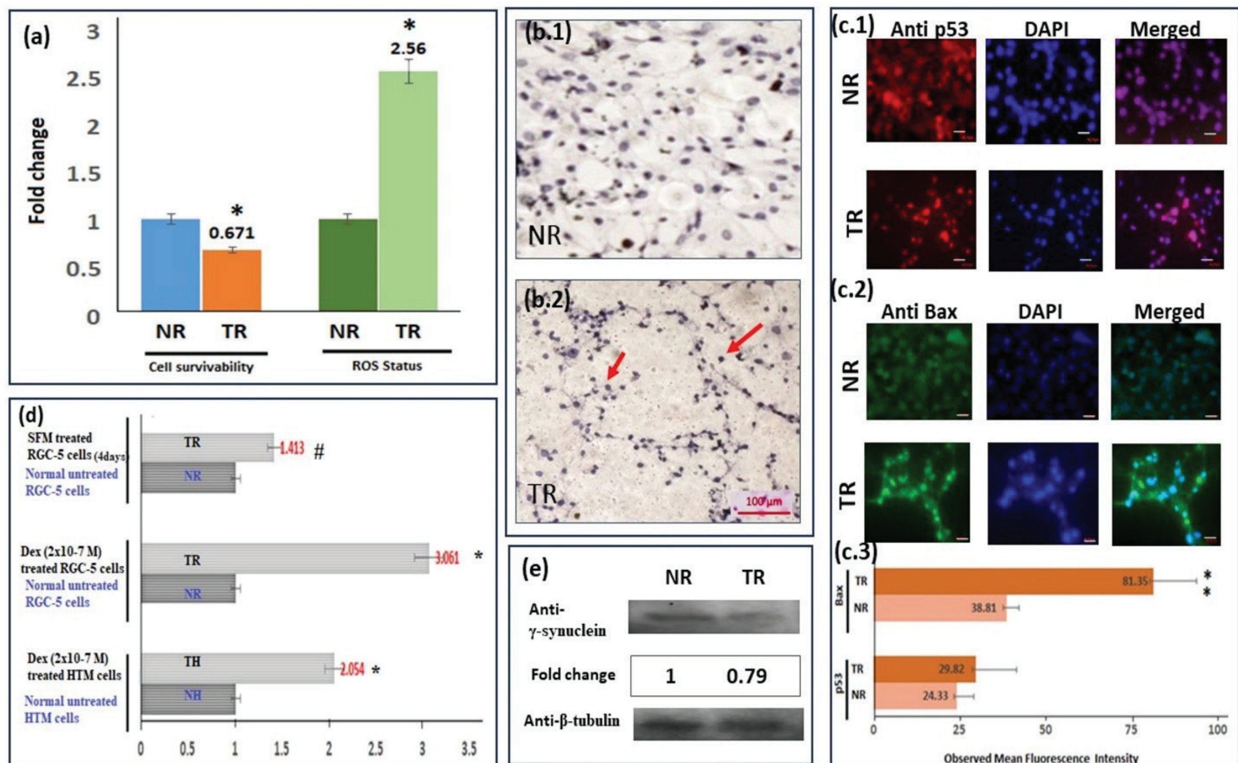

Figure 2: Effect of serum free medium (SFM) on RGC-5cells

a - Cell viability (MTT assay) and ROS status (H_2DCFDA assay); b.1 & b.2 - HE staining; c.1 - imageJ analysis for pro-apoptotic markers p53 and c.2 - Bax; c.3 - observed mean fluorescence intensity; d - myocilin expression in HTM and RGC-5 cells; e - γ-synuclein expression.

NH: normal untreated HTM cells; TH: dexamethasone treated HTM cells; NR: normal untreated RGC-5 cells; TR: dexamethasone treated RGC-5 cells

* represents significant change at $p < 0.05$; ** represents significant change at $p < 0.01$; # represents significant change at $p < 0.1$).

Scale bar represent 10 µm.

with upregulation of myocilin and downregulation of γ-synuclein. This suggests that dexamethasone, upon continuous administration, may be affecting HTM cells first by apoptosis and subsequently RGCs by slowly depleting their nutrient supply, leading to their apoptotic loss. Upregulated myocilin and downregulated γ-synuclein represent very specific and confirmatory glaucoma-associated features (Figure 3).

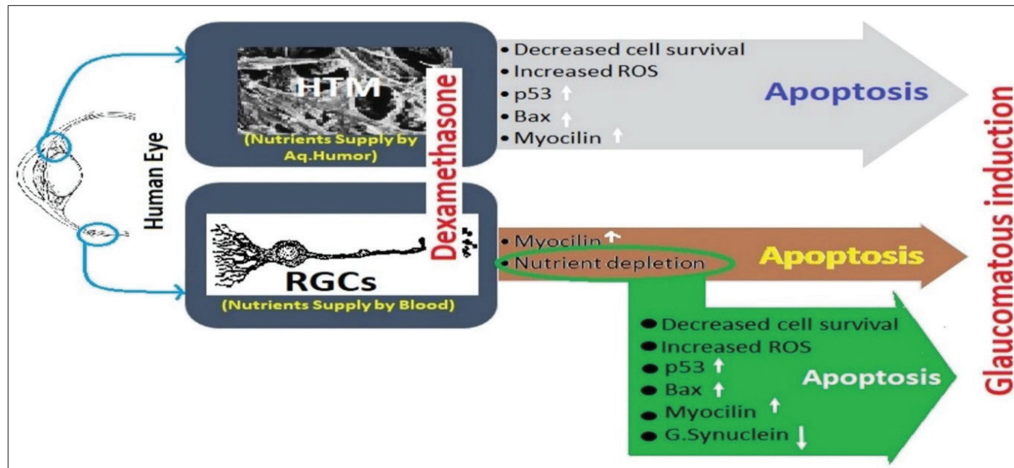

Figure 3: Overview of glaucomatous process in HTM and RGCs upon dexamethasone challenge

CONCLUSION

On the basis of observations in the present study, it can be concluded that the two crucial ocular parts TM and RGCs have been affected by dexamethasone in different ways. The HTM cells show apoptotic induction upon dexamethasone exposure directly while the RGCs get affected indirectly as dexamethasone causes increase in IOP that leads to nutrient depletion. This nutrient deprived condition is simulated by SFM treatment of the RGCs and in this condition, these cells show apoptotic symptoms. Thus, upregulation of myocilin in dexamethasone and SFM treatment conditions in the HTM and RGCs takes place along with downregulation of γ-synuclein in the RGCs upon SFM treatment.

Acknowledgement

This study was supported by the grants from DST-PURSE program of Jawaharlal Nehru University (JNU), New Delhi, India and University Potential of Excellence II under University Grant Commission India, towards JNU. The authors acknowledge fellowship agencies, Indian Council of Medical Research (ICMR) for financial support. Overall financial assistance is gratefully acknowledged to the University Grants Commission, Department of Science & Technology, Department of Biotechnology, Indian Council of Medical Research and Council of Science and Industrial Research, Govt. of India.

REFERENCES

Alagoz G., Gurel K., Bayer A., Serin D., Celebi S. & Kukner S. (2008). A comparative study of bimatoprost and travoprost: effect on intraocular pressure and ocular circulation in newly diagnosed glaucoma patients. *Ophthalmologica* **222**: 88–95.
DOI: https://doi.org/10.1159/000112624

Bernstein H.N. (1962). Schwartz B. Effects of long-term systemic steroids on ocular pressure and tonographic values. *Archives of Ophthalmology* **68**: 742–753.
DOI: https://doi.org/10.1001/archopht.1962.00960030746009

Chin E.K. *et al*. (14 authors) (2017). Ocular hypertension after intravitreal dexamethasone (ozurdex) sustained-release implant. *Retina* **37**(7): 1345–1351.
DOI: https://doi.org/10.1097/IAE.0000000000001364

Findl O., Strenn K., Wolzt M. Menapace R., Vass C, Eichler H.G. & Schmetterer L. (1997). Effects of changes in intraocular pressure on human ocular haemodynamics. *Current Eye Research* **16**: 1024–1029.
DOI: https://doi.org/10.1076/ceyr.16.10.1024.9024

Ge J., Wu Y., Qiu P., Liu H. & Lin M. (1999). Effect of N-methyl-D-aspartate and dexamethasone on apoptosis of rat cultured retinal ganglion cells. *Yan Ke Xue Bao* **15**(2):

65–69.

Goldmann H. (1962). Cortisone glaucoma. *Archives of Ophthalmology* **68**: 621–626.
DOI: https://doi.org/10.1001/archopht.1962.00960030625009

Gruver-Yates A.L. & Cidlowski J.A. (2013). Tissue-specific actions of glucocorticoids on apoptosis: A double-edged Sword. *Cells* **2**(2): 202–223.
DOI: https://doi.org/10.3390/cells2020202

Kasetti R.B., Maddineni P., Patel P.D., Searby C., Sheffield V.C. & Zode G.S. (2018). Transforming growth factor β2 (TGFβ2) signaling plays a key role in glucocorticoid-induced ocular hypertension. *Journal of Biological Chemistry* **293**(25): 9854–9868.
DOI: https://doi.org/10.1074/jbc.RA118.002540

Liu W., Zhao Z., Na Y., Meng C., Wang J. & Bai R. (2018). Dexamethasone-induced production of reactive oxygen species promotes apoptosis via endoplasmic reticulum stress and autophagy in MC3T3-E1 cells. *International Journal of Molecular Medicine* **41**: 2028–2036.
DOI: https://doi.org/10.3892/ijmm.2018.3412

Matsuda A., Asada Y., Takakuwa K., Sugita J., Murakami A. & Ebihara N. (2015). DNA methylation analysis of human trabecular meshwork cells during dexamethasone stimulation. *Investigative Ophthalmology and Visual Science* **56**(6): 3801–3809.
DOI: https://doi.org/10.1167/iovs.14-16008

Oshima Y., Kuroda Y., Kunishige M., Matsumoto T. & Mitsui T. (2004). Oxidative stress-associated mitochondrial dysfunction in corticosteroid-treated muscle cells. *Muscle Nerve* **30**: 49–54.
DOI: https://doi.org/10.1002/mus.20036

Patel G.C., Phan T.N., Maddineni P., Kasetti R.B., Millar J.C., Clark A.F. & Zode G.S. (2017). Dexamethasone-induced ocular hypertension in mice: effects of myocilin and route of administration. *The American Journal of Pathology* **187**(4): 713–723.
DOI: https://doi.org/10.1016/j.ajpath.2016.12.003

Pereiro X., Ruzafa N., Acera A., Fonollosa A., Rodriguez F.D. & Vecino E. (2018). Dexamethasone protects retinal ganglion cells but not Müller glia against hyperglycemia in vitro. *PLoS One* **13**(11): e0207913.
DOI: https://doi.org/10.1371/journal.pone.0207913

Resch Z.T. & Fautsch M.P. (2009). Glaucoma-associated myocilin: a better understanding but much more to learn. *Experimental Eye Research* **88**(4): 704–712.
DOI: https://doi.org/10.1016/j.exer.2008.08.011

Sato K., Nishiguchi K.M., Maruyama K., Moritoh S., Fujita K., Ikuta Y., Kasai H. & Nakazawa T. (2016). Topical ocular dexamethasone decreases intraocular pressure and body weight in rats. *Journal of Negative Results in Biomedicine* **15**: 5.
DOI: 10.1186/s12952-016-0048-x

Sayyad Z., Sirohi K., Radha V. & Swarup G. (2017). 661W is a retinal ganglion precursor-like cell line in which glaucoma-associated optineurin mutants induce cell death selectively. *Scientific Reports* **7**: 16855.
DOI: https://doi.org/10.1038/s41598-017-17241-0

Surgucheva I., Shestopalov V. & Surguchov A. (2008). Effect of synuclein silencing on apoptotic pathways in retinal ganglion cells. *Journal of Biological Chemistry* **283**(52): 36377–36385.
DOI: 10.1074/jbc.M806660200

Tamm E.R., Russell P., Epstein D.L., Johnson D.H. & Piatigorsky J. (1999). Modulation of myocilin/TIGR Expression in human trabecular meshwork. *Investigative Ophthalmology and Visual Science* **40**: 2577–2582.

Tamm E.R. (2002). Myocilin and glaucoma: facts and ideas. *Progress in Retinal and Eye Research* **21**(4): 395–428.
DOI: https://doi.org/10.1016/s1350-9462(02)00010-1

You J.M., Yun S.J., Nam K.N., Kang C., Won R. & Lee E.H. (2009). Mechanism of glucocorticoid-induced oxidative stress in rat hippocampal slice cultures. *Canadian Journal of Physiology and Pharmacol*ogy **87**: 440–447.
DOI: https://doi.org/10.1139/y09-027

World Health Organization (2013). *18th WHO Model List of Essential Medicines*. Available at *https://www.who.int/medicines/publications/essentialmedicines/18th_EML.pdf*

Zhuo Y.H., He Y., Leung K.W., Hou F., Li Y.Q., Chai F. & Ge J. (2010). Dexamethasone disrupts intercellular junction formation and cytoskeleton organization in human trabecular meshwork cells. *Molecular Vision* **16**: 61–71.