

# ALTERATION IN MATRIX METALLOPROTEINASES (MMPS) ACTIVITY IN FIBROBLAST CELL LINE BY DEXAMETHASONE: A POSSIBLE MECHANISM IN CORTICOSTEROID-INDUCED GLAUCOMA

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## ABSTRACT

Corticosteroids are often used as anti-inflammatory agents in a variety of inflammatory diseases. It is well established that long-term administration of corticosteroids predisposed the patients to develop glaucoma. Although the exact pathophysiology of steroid-induced glaucoma is unknown, it is assumed that Matrix metalloproteinases (MMPs) have a role in its pathogenesis.

To study and estimate the pathophysiological effects of MMPs in glaucoma, we established an in vitro cell culture model. We also employed a precise proliferation assay to analyze cytotoxic effect of dexamethasone. The influence of dexamethasone on MMPs production was investigated using an in vitro gelatin Zymography.

Cytotoxicity analysis of Dexamethasone revealed no significant cell death in low concentration. However, it caused 50% and 70% cell death at 80 and 100 µg/mL respectively. It also revealed an inhibitory effect on MMPs by dexamethasone in a dose dependent fashion.

It may be concluded that an alteration in the level of MMPs expression by dexamethasone interferes with ocular fluid drainage and may contribute to the pathogenesis of glaucoma.

**Keywords:** Matrix metalloproteinase, Intraocular pressure, Cytotoxicity, Corticosteoid.

## INTRODUCTION

Corticosteroids are one of the most potent and effective modalities available in the treatment of many inflammatory diseases. However, they can produce a plethora of adverse ocular and systemic events.<sup>1-4</sup> Glau-

coma is one of the unwanted effects of the administration of these drugs. Glaucoma is now defined as a group of ocular diseases characterized by elevated intraocular pressure (IOP) that causes progressive damage to the optic nerve, resulting in optic nerve atrophy and eventual blindness.<sup>5-7</sup> Based on the anatomy of the anterior chamber of the eye, which is filled with the aqueous humor, the two primary types of diseases, angle-closure glaucoma and open-angle glaucoma, are

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defined. Open-angle glaucoma accounts for at least two third of cases of glaucoma. Primary risk factors are age, race, family history of glaucoma, high myopia, diabetes mellitus and corticosteroid especially in high dosage or prolonged use.<sup>8-10</sup> In the eyes, maintenance of IOP is dependent on a balance between aqueous formation and aqueous outflow. The aqueous percolates through iridocorneal junction organized by trabecular meshwork (TM). The major component of TM is extracellular matrix (ECM).<sup>11-13</sup> Most investigators unanimously admitted that matrix metalloproteinases (MMPs) were critical enzymes in the alteration of ECM.<sup>14,15</sup>

MMPs are a family of highly homologous, zinc and calcium dependent endopeptidase that cleave most, if not all, components of ECM. Among members of the family of human MMPs, Gelatinase A and B (MMP2 and 9) degrade basement membrane collagens type IV, gelatin and other proteoglycan component of the extracellular matrix.<sup>16-18</sup>

As the mechanisms underlying alteration of iridocorneal angle in corticosteroid-induced glaucoma have not been precisely described, here we employed a cellular model to study the probable influence of corticosteroid on activity of matrix metalloproteinases (MMPs) by enzyme zymography as well as its cytotoxic effects, in order to investigate its possible mechanism in corticosteroid-induced glaucoma.

## MATERIALS AND METHODS

### Cell culture

The cell, WEHI 164, was seeded at initial density of  $2 \times 10^4$  cells/well in 96-well tissue culture plates. Cells were maintained in RPMI-1640 medium supplemented with 5% fetal calf serum, penicillin at 100 units/ml, and streptomycin at 100  $\mu$ g/ml, under 5% CO<sub>2</sub>, 37°C and saturated humidity.

### Dose-response analysis

Triplicate, two-fold dilutions of dexamethasone was transferred to overnight cultured cells. Non-treated cells were used as control. Cells were cultured overnight and were then subjected to colorimetric assay. A sample of the media was used for zymoanalysis.

### Colorimetric assay

After each experiment, the cells were washed three times with ice-cold PBS, followed by fixation in a 5% formaldehyde solution. Fixed cells were washed three times and stained with 1% crystal violet. Stained cells were washed, lysed and solubilised with 33.3% acetic acid solution. The optical density of developed purple color of solution was read at 580 nm.

### Zymoanalysis

This technique has been used for the detection of gelatinase (collagenase type IV or matrix metalloproteinase type 2, MMP-2) and MMP-9, in conditioned-media according to Heussen and Dowdle method (19) with some modifications. Briefly, aliquots of conditioned media were subjected to electrophoresis in (2mg/ml) gelatin containing polyacrylamide gels, in the presence of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under non-reducing conditions. The gels underwent electrophoresis for 3 hours at a constant voltage of 80 volts. After electrophoresis, the gels were washed and gently shaken in three consecutive washings in 2.5% Triton X100 solution to remove SDS. The gel slabs were then incubated at 37°C overnight in 0.1 M Tris HCl gelatinase activation buffer (pH 7.4) containing 10mM CaCl<sub>2</sub> and subsequently stained with 0.5% Coomassie Blue. After intensive destaining, proteolysis areas appeared as clear bands against a blue background. Using a UVI Pro gel documentation system (GDS\_8000 System), quantitative evaluation of both surface and intensity of lysis bands, on the basis of grey intensity, were compared relative to non-treated control wells and expressed as "Relative Expression" of gelatinolytic activity.

### Statistical analyses

The differences in cell proliferation and gelatinase activity were compared using the Student's t test. P values <0.05 were considered significant.

## RESULTS

The cytotoxic effects of dexamethasone were not significant in a low concentration. By contrast, it caused 50% and 70% cell death at 80 and 100  $\mu$ g/mL respectively. Cell cytotoxicity of dexamethasone is illustrated in Figure 1.

As shown in Fig. 2, treated cultured cells with different concentration of dexamethasone were subjected to gelatin-A zymography. The relative expression of MMPs activity of dexamethasone is presented in figure 3; the inhibitory activities were observed at all concentrations

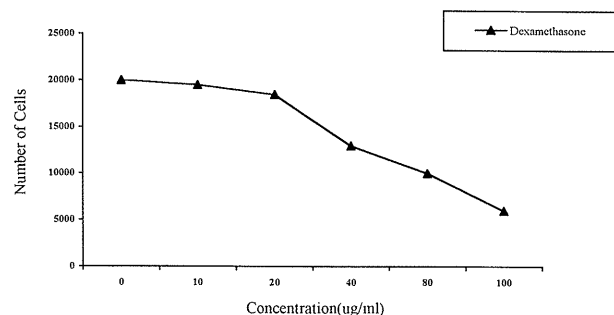


Fig. 1. Cytotoxicity analysis of Dexamethasone on in vitro cellular model.

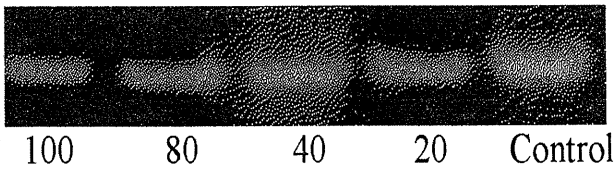


Fig. 2. Zymograph of Dexamethasone in descending concentrations.

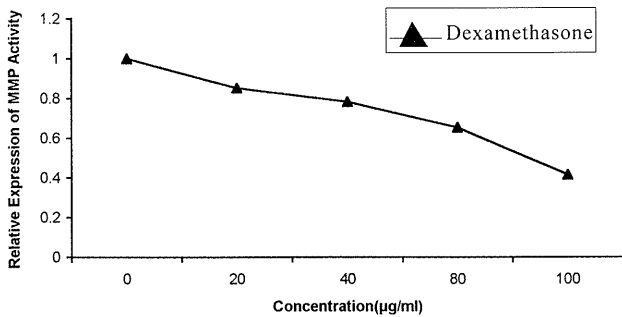


Fig 3. Dose response analyses of Dexamethasone on MMPs relative activity.

in a dose-response fashion.

### DISCUSSION

Corticosteroid-induced glaucoma has partly been described by alteration in ECM remodeling like many other pathological conditions.<sup>20-31</sup> In addition, one of the critical steps for ocular fluid drainage is the destruction of ECM, which is catalyzed mainly by the MMPs.<sup>32</sup> However, the exact pathophysiology of corticosteroid-induced glaucoma is controversial. Therefore, studies regarding the effects of corticosteroid agents, especially in terms of their stimulatory or inhibitory effect on the biosynthesis of extracellular matrix components can be beneficial. Our zymography analysis revealed similar results with some previous studies on the effects of the steroidal anti-inflammatory drugs on MMPs inhibition. The observed reduction of MMPs activities, however, was associated with increasing concentration of this drug.<sup>33-35</sup> In addition, cytotoxicity analysis of dexamethasone showed significant decreases in cell number in concentration up to 40 µg/mL. Taken together, it might be argued that both of these observed effects may lead to glaucoma through enzymatic ECM destruction as well as alteration of trabecular meshwork (TM) of iridocorneal angle, thus culminating in altered ocular fluid drainage.

Although, not all patients receiving corticosteroids would develop elevated IOP, patients, particularly children, on chronic corticosteroid therapy could remain undiagnosed with an elevated IOP.<sup>36</sup> This undiagnosed IOP may result in glaucomatous optic nerve damage.

Therefore, it would be beneficial for patients under corticosteroid therapy to be monitored for the possibility of developing glaucoma.

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