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Original Research

Evaluation of Corneal Histopathologic Changes in Rabbits Due to Topical Mitomycin C Application in Different Doses and Periods

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ABSTRACT

Purpose: To evaluate the histopathological changes in the cornea owing to topical mitomycin C (MMC) application in various doses and administration periods.

Methods: The study group consisted of 35 albino rabbits with a mean age of 6 months. The animals were divided into the 7 groups, with each group comprising 10 eyes of 5 rabbits. Five rabbits were used as the control group, and the others were divided into groups according to the application period. The first 3 groups subsequently underwent 0.2, 0.4, and 0.8 mg/mL mitomycin C application 6 times at weekly intervals. Groups 4, 5, and 6 underwent MMC application at the same doses 12 times. The control group underwent topical physiological serum application. After the last treatment, the rabbits were sacrificed and enucleation was performed.

Results: Light and electron microscopic examinations of MMC-treated animals revealed different histopathological changes in the corneal epithelium and endothelium, according to various doses and administration periods.

Conclusion: Topical MMC has toxic effects on the cornea related to dose and period, and it is known to be more toxic in increased doses. In this study, we identified the most effective dose without side effects. Long-term treatment with MMC at low doses is more advantageous than short-term treatment at high doses.

Keywords: Cornea, topical mitomycin C, toxic effect

INTRODUCTION

Mitomycin C (MMC) is a chemotherapeutic agent with antifibrinolytic and antineoplastic features, derived from Streptomyces caespitosus (1). MMC is used topically for the treatment of ocular surface tumors, glaucoma, pterygium, lacrimal canal, and ocular cicatrization. Moreover, MMC used as an adjuvant therapy after corneal refraction surgery and has a limited use in vernal keratoconjunctivitis, lacrimal drainage system tumors, orbital implant, proliferative vitreoretinopathy, and cataract surgery.

Generally MMC is used topically for a short period in patients undergoing photorefractive keratectomy (PRK) for myopia to prevent haze formation. It also may be used for longer periods in patients with ocular surface tumors but may cause side effects. Because of its potential serious and irreversible side effects, such as scleral melting and limbal stem cell deficiency, the application form, period, and dose vary according to physician (2). In the present study, we aimed to evaluate the histopathological changes in the cornea of rabbits caused by treatment with topical MMC at various doses and application periods and to determine the most effective dose without toxic effects to prevent its side effects.

METHODS

This study protocol was approved by the Animal Ethics Review Committee of the Çukurova University Medical Faculty. The study group consisted of 35 New Zealand albino rabbits weighing 2961±424.7 g (range, 2020-3860 g) with a mean age of 6 months. The animals were divided into 7 groups, each consisting of 10 eyes of 5 rabbits. Five rabbits were used as the control group (Group 0) and underwent topical physiological serum application at weekly intervals. Groups 1, 2, and 3 received 0.2, 0.4, and 0.8 mg/mL of MMC (Kyowa Kirin Ltd), respectively, 6 times at weekly intervals (1 week with and 1 week without drop application). Groups 4, 5, and 6 received MMC at the same doses, but 12 times in weekly intervals, similar to the topical MMC treatment model. Tumor cells with proliferative features were more sensitive to this type of therapy, whereas normal cells had time for repair. Thus, the intensity of adverse effects is believed to be lower (Table 1). After the final treatment, all rabbits were sacrificed and enucleation was performed.

RESULTS

Light microscopic examination of the corneas in the control group cornea revealed a regular arrangement (Figure 1). Fur-

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Table 1. The properties of the groups

	Number of eyes (n)	Duration	Number of cures
Group 0 (Control)	10	3 months	6
Group 1	10	3 months	6
Group 2	10	3 months	6
Group 3	10	3 months	6
Group 4	10	6 months	12
Group 5	10	6 months	12
Group 6	10	6 months	12

Figure 1. Regular arrangement of cornea epithelium and cytoplasm was observed in group 0 (control group) (hematoxylin and eosin staining, ×400)

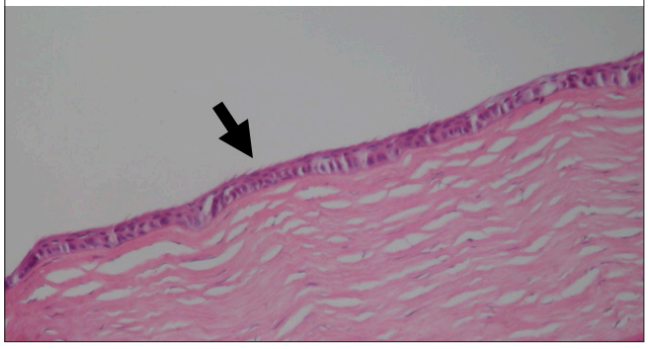
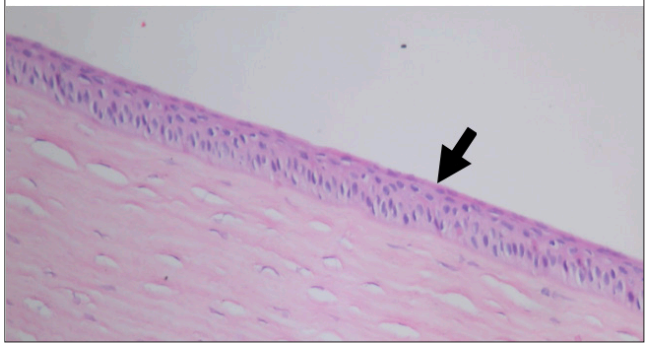


Figure 2. Increase in the linear arrangement of the cornea epithelium in group 6 (dose, 8 mg/mL; treatment duration, 6 mo; number of cures, 12) (hematoxylin and eosin staining, ×400)



Main Points:

- MMC is a preferred therapeutic agent for ophthalmological disorders owing to its antineoplastic and antifibrotic features.
- Because of its potential serious and irreversible side effects, the application form, period, and dose vary according to physician.
- In terms of side effects, long-term treatment with MMC at low doses is more advantageous than short-term treatment at high doses.

thermore, an increase in the number of epithelial cells, epithelial thickening, and cellular loss were noted (Figure 2).

The cornea endothelium showed a regular arrangement and insignificant cytoplasm (Figure 3). Endothelial cell thinning, superficial microvillus loss, cytoplasmic vacuolization, and cellular loss were the most common changes observed in the endothelium (Figure 4). These changes were found to be greater according to an increase in dose and application period. An increase in dose was found to be more effective in the development of these findings.

Light microscopic evaluation was graded as follows: 0 (none), 1 (mild), 2 (intermediate), and 3 (intensive). Evaluation of the affection ratio of the groups revealed that dose was a more effective parameter than the application period (Table 2).

Electron microscopic examination of the corneal epithelium revealed nonkeratinized squamous epithelial cells and short microvillus at the apical surface of the corneal epithelial cells

Figure 3. In group 0 (control group), cornea endothelium presented with a regular arrangement and insignificant cytoplasm (hematoxylin and eosin staining, ×400)

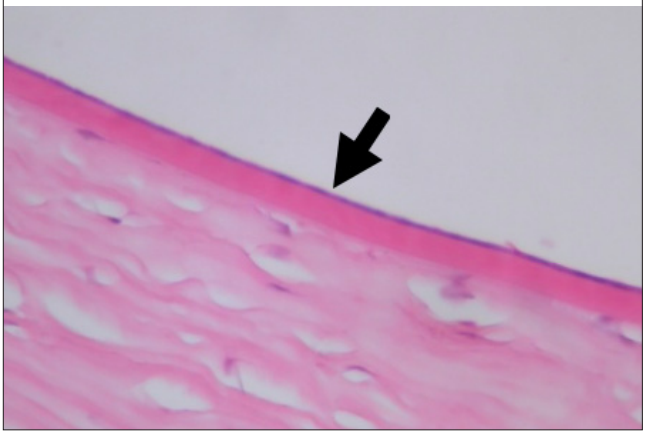


Figure 4. Vacuolar degeneration was observed in the cytoplasm of the corneal endothelium in group 6 (dose, 0.8 mg/mL; duration of treatment, 6 mo; number of cures,12) (hematoxylin and eosin staining, ×400)

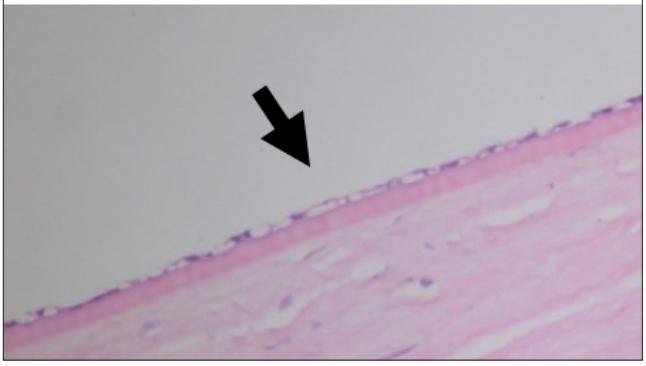


Table 2. Increase in affection owing to increased dose and administration period and effect of the administration of a higher dose

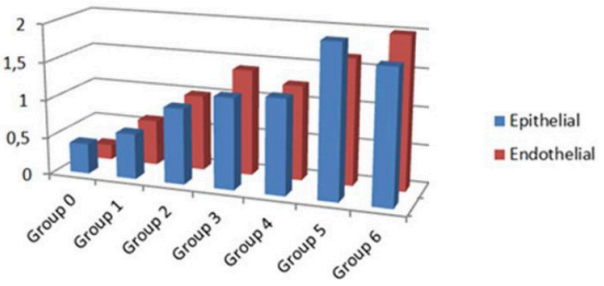


Figure 5. In group 0 (control group), the corneal epithelium comprised nonkeratinized squamous epithelial cells. Short microvilli at the apical surface of the corneal epithelial cells (arrows) and intercellular binding complexes (white arrows) can be seen. N, nucleus

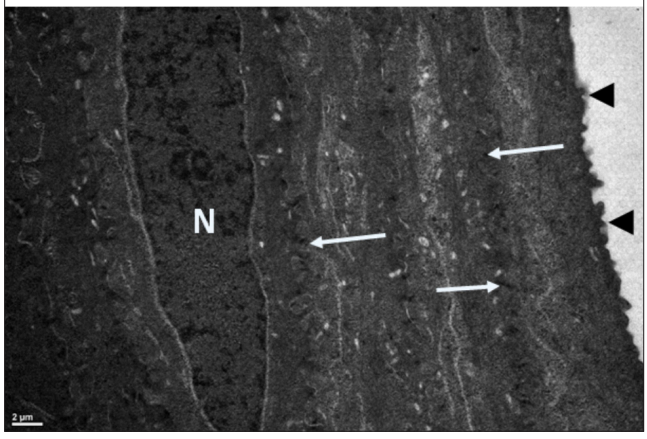
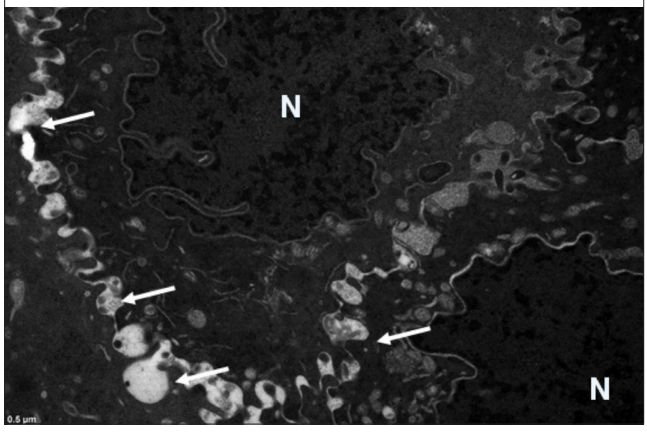


Figure 6. Cornea from group 6 (duration of treatment, 6 mo; dose, 0.8 mg/mL). Intercellular expansion presenting with edema in the cornea superficial epithelium (white arrows). N, nucleus



(Figure 5). Furthermore, the following findings were observed: expansion of the intercellular area owing to edema, presence of electrodense epithelial cells, a decrease in intracellular binding complexes, and an increase in intercellular distance. An increase in the amount of chromatin in the cell nuclei, deep indentations, expansion in the mitochondria of the cytoplasm, and vacuolization were frequent findings. Except for mild expansion in fibroblasts, the stroma was found to be normal (Figure 6). The endothelial cell layer and microvilli at the apical surface of the endothelial cells were also normal (Figure 7). Thinning in the corneal endothelial cells, superficial microvillus loss, and cytoplasmic vacuolization were common findings (Figure 8). These findings varied according to the dose and application period of MMC.

Figure 7. Endothelial cell layer (En) from group 0 (control group). Microvilli at the apical surface of the endothelial cells (black arrow). N, nucleus; D, Descemet membrane

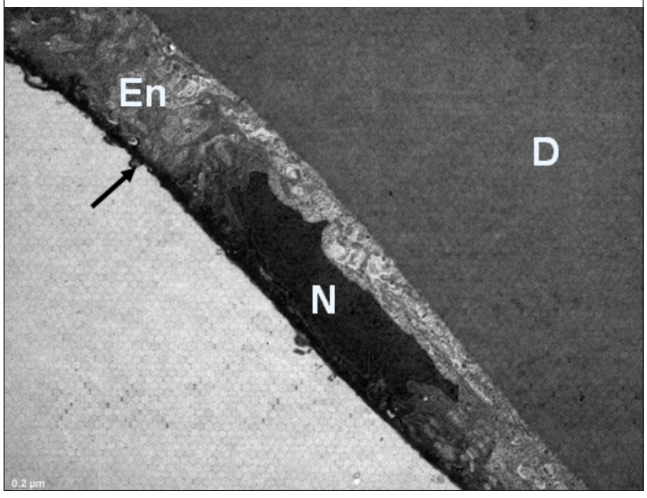
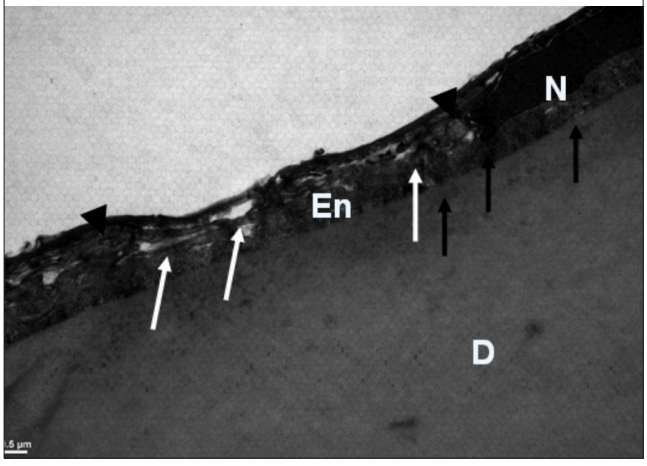


Figure 8. Cornea from group 6 (duration of treatment, 6 mo; dose, 0.8 mg/mL). Extensive expansion (white arrow) between the endothelial cells (En) presenting with edema. Decrease in the number of microvilli on the apical surface of the endothelial cells (arrows) and mild vacuolization (black arrows). N, nucleus; D, Descemet membrane



DISCUSSION

MMC is a preferred therapeutic agent for ophthalmological disorders owing to its antineoplastic and antifibrotic features. It is widely used in filtering glaucoma surgery because of its antifibrotic effects, and it is used in ocular surface tumors and pterygium surgery because of its antineoplastic effects. However, there is no consensus regarding the dose and application period of MMC because of its irreversible side effects on normal cells.

Mohan et al. (3) used the terminal deoxyribonucleotidyl transferase-mediated dUTP-digoxigenin nick end labeling (TUNEL) system to determine the effects of the MMC application period and concentration of MMC on corneal apoptosis. They observed a significant increase in the TUNEL- positive stained cells in high doses. However, the increase resulting from long-term treatment was not statistically significant. Furthermore, Mohan et al. (3) detected maximal apoptosis at the fourth hour after photoreactive keratectomy.

Gharaee et al. (4) reported that the administration of 0.02% MMC over the ablated area for 5 seconds for each diopter of spherical equivalent in PRK can affect the morphology of the endothelial cells, but it does not change the cell density.

After a 6-month follow-up period, Zare et al. (5) reported that there were no significant changes in the endothelial cell density and morphology of the cornea after the administration of topical MMC during PRK.

Shojaee et al. (6) reported no significant change in the endothelial cell density of the cornea after the administration of topical MMC during PRK. The postoperative haze grade was notably lower in the MMC group.

Light microscopic examination of the exenteration material from 2 patients with conjunctival melanoma who underwent 0.4 mg/mL topical MMC treatment for 7 and 28 days subsequently did not reveal toxic effects on the episclera, cornea, iris, ciliary body, lens, or retina (7). However, the findings of that study were limited by a short-term application of MMC and evaluation under light microscopy, which is not as sensitive as electron microscopy. Therefore, minimal changes may not be observed using this method.

Avisar et al. (8) applied 0.2 mg/mL of topical MMC for 5 minutes during pterygium surgery. The preoperative endothelial cell count of 2254±128 cells/mm^2 decreased 21.25%±2.8% in the first postoperative week, 24.26%±1.8% in the first month, and 21.05%±3.2% by the third month. Consequently, the difference in the values was significant at all time points.

We evaluated the effects of MMC on the cornea under light microscopy with hematoxylin and eosin and observed a significant linear increase in epithelial cell numbers in groups that received a high number of doses and those that received a high dose of MMC for a long period of time (0.4 vs 0.8 mg/mL). These findings were not prominent at lower doses. Compared with the control group, the severity of vacuolar degeneration and cellular disarrangement in the corneal endothelial cells of the treatment group was correlated with a high dose and short administration period. Electron microscopic examination of the epithelium revealed enlargement of intercellular areas resulting from edema, presence of electrodense cells, and a decrease in intercellular binding complexes. Increases in nuclear chromatin, profound indentations, expansion of mitochondria, and cytoplasmic vacuolization were also observed. The stroma was normal except for expansion in the fibroblasts. Thinning of the endothelial cells, loss of superficial microvilli, and cytoplasmic vacuolization were common findings. The severity of these findings was also in accordance with the dose and administration period of MMC.

The cornea of rabbit differs from that of humans in that the former does not have the Bowman membrane and is, thus, thinner. In addition, the corneal endothelial cells of rabbits reproduce via mitosis. Therefore, the results obtained in rabbits cannot be generalized to humans.

MMC is markedly effective on proliferative cells. However, the cornea of rabbits is more sensitive because it is thin and exhibits mitotic activity in the endothelium. The risk of endothelial failure is extremely low because the damaged endothelium can be repaired by mitosis (9, 10).

Because of the antineoplastic properties of MMC, it is widely used as an antiproliferative agent for tumor therapy and surgical interventions. However, severe and irreversible side effects may develop after the application of MMC soaked sponges in glaucoma and pterygium surgery. In individuals with ocular surface tumors, long-term administration of MMC can cause side effects. Therefore, studies on agents with antineoplastic versus antifibrotic effects are ongoing. Among them, the most important studies are those on fluorouracil and interferon-2b. The adverse effects of these agents appear to be less than those of MMC. However, more studies must be conducted to confirm this hypothesis (11).

In our study, long-term administration of MMC at low doses produced cytotoxic effects in the cornea. However, these findings were severe in groups that received high doses (0.8 mg/mL). Short-term high dose treatment resulted in severe histopathological changes in the cornea compared with long-term low-dose treatment. Therefore, the most effective low-dose of topical MMC should be administered to prevent irritation and possible cytotoxic effects. This will prevent the loss of limbal stem cells, punctal stenosis, ocular irritation, conjunctival hyperemia, lacrimation, punctate keratopathy, blepharospasm, corneal edema, and ocular pain owing to topical MMC administration. Several studies have assessed various doses and methods. Treatment with low-dose MMC (0.02 mg/mL) for primary and recurrent ocular surface tumors was found to result in tumor regression without any side effects (12).

Currently, MMC is widely used in ophthalmology, and its side effects on the cornea, particularly with high dose treatments, are well known. Long-term, low-dose treatment with MMC may be more advantageous than short-term, high dose treatment in

terms of preventing side effects. Therefore, the former should be preferred. MMC is also used in refractive surgery, where these side effects have also been observed. In non-regressed ocular surface tumors, the number of cures should be increased. Furthermore, a decrease in the current concentration will facilitate better tolerance of the drug.

It remains unclear whether histopathological changes in the cornea resulting from MMC treatment are reversible or permanent. We did not evaluate histopathological changes after the termination of treatment. However, studies should be conducted to address this issue and to clarify the safe and effective MMC dose and treatment duration. There is not currently a consensus regarding MMC dose and treatment duration. Because of the severe side effects of treatment with topical MMC, surgery appears to be the first-line treatment for ocular surface tumors. Therefore, MMC is administered in inactive doses or is discontinued early.

This study aimed to determine the adequate application period for the lowest active dose and the occurrence of side effects in these circumstances. We evaluated the histopathological changes in the cornea caused by treatment with topical MMC at various doses and application periods. Significant changes in the cornea were observed in the high- dose MMC treatment groups.

CONCLUSION

In terms of side effects, long-term, low-dose treatment with topical MMC is more preferred than short-term, high dose treatment. Therefore, the former should be recommended.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Animal Ethics Review Committee of the Çukurova University School of Medicine (28.11.2007- No: 22).

Peer-review: Externally peer-reviewed.

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Conflict of Interest: Authors have no conflicts of interest to declare.

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