Ultrastructural Changes in Rabbit Ciliary Body after Extraocular Mitomycin C

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PURPOSE. To study the ultrastructural changes in ciliary body epithelium of the rabbit eye after subconjunctival injections of mitomycin C.

METHODS. One eye of six New Zealand white rabbits was given a subconjunctival injection at the 12-o'clock position with 0.005, 0.02, 0.08, 0.1, 0.12, or 0.16 mg mitomycin C. The fellow eye was given a subconjunctival injection of balanced salt solution. Two weeks after treatment, the eyes were enucleated, and the ciliary body was exposed and submerged in fresh 4% paraformaldehyde/2% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, at 4°C. Electron microscopy of the ciliary body was performed at two sites: the injection site (12-o'clock position) and 180° away (6-o'clock position).

RESULTS. At dosages of 0.1 mg and higher, ciliary body epithelial cells beneath the injection site were thinned. There were vacuoles and expansion of intracellular and intercellular spaces. Plasma membrane infoldings were disrupted, and the apical membrane was thinned. Mitochondria and nuclei were normal. Ciliary body epithelium at 6-o'clock position on the eye of either 0.05, 0.20, 0.80, 1.0, 1.2, or 1.6 mg/ml MMC. The fellow eye was given a 0.1-ml injection of balanced salt solution showed normal ciliary body epithelium at the injection site and 180° away.


Mitomycin C (MMC), an antibiotic isolated from Streptomyces caesipitosus with antiproliferative activity against fibroblasts, has gained popularity as adjunctive therapy during glaucoma filtration surgery. Proposed mechanisms of improved success with MMC filtration surgery include decreased resistance to outflow with thinner blebs and cytotoxic ciliary body effects leading to decreased aqueous humor production. Extraocular application of MMC in the rabbit model has produced contradictory results. Some investigators have demonstrated direct cytotoxic effects of MMC on the ciliary body epithelium, whereas others have not. Given the frequency of MMC use during trabeculectomy and the higher incidence of hypotony after filtration surgery with this agent, we sought to delineate more clearly the possible role of MMC in ciliary body damage.

METHODS

Mitomycin C (Bristol Myers Squibb Oncology, Princeton, New Jersey) was reconstituted in sterile balanced salt solution. One eye from each of six New Zealand White rabbits was given a 0.1-ml subconjunctival injection at the 12-o'clock position on the eye of either 0.05, 0.20, 0.80, 1.0, 1.2, or 1.6 mg/ml MMC. The fellow eye was given a 0.1-ml injection of balanced salt solution. The rabbits were anesthetized with ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (5 mg/kg) before subconjunctival injections. All handling of the rabbits was in compliance with the University of Virginia Animal Research Committee Protocol and the ARVO Guidelines on Use of Animals in Ophthalmic and Vision Research. Tobramycin and dexamethasone sterile ophthalmic solutions were instilled in each eye 4 times a day for 1 week. At the end of 2 weeks, the rabbits were euthanized. All treated and control eyes were enucleated. Immediately after enucleation, the eyes were bisected to maximally expose the ciliary bodies and submerged in fresh 4% paraformaldehyde/2% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, at 4°C. After fixation, segments of the ciliary body at the injection site superiorly and 180° away (6-o'clock position) were dissected and routinely processed for electron microscopy through 2% osmium tetroxide, ethanol dehydration, and infiltration and embedding in epoxy resin. Ultrathin sections (70-80 nm in thickness) were counterstained with lead citrate and uranyl acetate and examined in an electron microscope (model JEOL 100-CX; Japan Electron Optics Limited, Tokyo, Japan).
FIGURE 1. Electron micrographs of control and mitomycin C (MMC)-treated rabbit ciliary body at injection site. (A) Control (balanced salt solution) illustrates normal ciliary body epithelial ultrastructure; 0.1 mg (B), 0.12 mg (C), and 0.16 mg (D) dose-related thinning of the apical membrane, disorganization of plasma membrane infoldings, and increased vacuolization. N, nucleus; arrows, apical membrane; arrowheads, plasma membrane infoldings; E, extravasated erythrocyte. Magnification, X16,250.
FIGURE 2. Low-magnification electron micrographs of rabbit ciliary body. (A) Injection site; 0.16 mg mytomycin C (MMC) showing marked abnormalities of the ciliary body epithelium. (B) An area 180° from injection site; same eye as in (A), showing normal ciliary body architecture. (C) Control; injection site; balanced salt solution. N, nucleus; arrows, apical membrane; arrowheads, plasma membrane infoldings; E, extravasated erythrocyte. Magnification, ×5000.
FIGURE 3. High-magnification electron micrographs of rabbit ciliary body treated with 0.16 mg mytomycin C (MMC). (A) Injection site showing marked thinning of apical membrane, disruption of plasma membrane infoldings, and vacuolization; (B) 180° from injection site same eye, showing normal ciliary body ultrastructure. n, nucleus; arrows, apical membrane; arrowheads, plasma membrane infoldings. Magnification, ×26,000.
RESULTS
At MMC dosages of 0.1 mg and higher, ciliary body epithelium in the region of the injection site was thinned compared with corresponding regions from control sites. There was diffuse cytoplasmic vacuolization in both pigmented and nonpigmented ciliary epithelial cells and expansion of intracellular and intercellular spaces (Fig. 1).

Infoldings of the plasma membrane of nonpigmented epithelial cells were disrupted. The glycocalyx-like apical membrane of the nonpigmented epithelial cells was thinned and appeared atrophic. The magnitude of these changes was more pronounced with increasing dosages of MMC (Fig. 1). Ciliary body epithelium located 180° away from the injection site showed only mild architectural distortion of the plasma membrane infoldings (Figs. 2, 3). Eyes that had received lower dosages of MMC (0.005 mg, 0.02 mg, and 0.08 mg) and contralateral control eyes showed normal ciliary body epithelium.

DISCUSSION
The lowered intraocular pressures found after MMC trabeculectomy may be a result of hyposecretion of aqueous humor, or a combination of the two. Potential cytocidal properties of MMC have been postulated. Letchinger et al. showed an intrinsic intraocular pressure-lowering effect of MMC in the rabbit model. Wilton et al. found that the intraocular pressure decreased with subconjunctival deposits of MMC in rabbits but not with topically applied MMC. Mietz and colleagues showed dense swollen mitochondria, increased vacuolization, and an electron-dense material near the endoplasmic reticulum of the nonpigmented ciliary epithelial cells of rabbits exposed to extraocular MMC. Nuyts et al. showed similar transmission electron microscopic changes after MMC trabeculectomy in human ciliary body epithelium after death. In contrast, Hollo and Suveges found no morphologic changes in the ciliary epithelium of rabbits after exposure to MMC.

In this study, we found evidence of direct ciliary body toxicity from subconjunctival injection of MMC. The nuclei and mitochondria were normal. We theorize that the normal appearance of the mitochondria helped to reduce the possibility that noted changes were secondary to tissue damage during enucleation and processing for electron microscopy. Damage was most noticeable at plasma membrane infoldings and at the glycocalyx-like apical membrane of the nonpigmented epithelium. These changes to the plasma membrane infoldings and apical membrane may have been a transient response to MMC. The epithelial cells might have recovered completely had we extended the length of the experiment.

Our results suggest that MMC diffused through the thin rabbit sclera to directly reach the ciliary body. MMC may also have been released from the subconjunctival injection site into the tear film and diffused through the cornea to reach the ciliary body. Had this latter route predominated, we would have expected to see more uniform damage to the ciliary body or more damage in the lower half of the eye where gravity would have directed MMC as it leaked out of the subconjunctival space. The normal appearance of the ciliary epithelium 180° away from the injection site in study eyes indicates that localized cytocidal destruction with MMC may be possible.

Human sclera is thicker than rabbit sclera. The thinner sclera of the rabbit may simulate the bed of a scleral flap made during trabeculectomy in humans. Although we left the MMC in the subconjunctival space and therefore exposed it to the sclera for longer than during routine trabeculectomy, we did not surgically breach the integument of the sclera. Had an intact sclera been a physical barrier to MMC, we would not have seen the localized changes to the ciliary body that were shown in this study. The thickness of a scleral flap, the placement of a MMC-soaked pledge on or under the flap, and the duration of time the pledge is left on the sclera may all determine how much MMC is gaining access to the ciliary body during trabeculectomy in humans.

In conclusion, subconjunctival injection of MMC appears to cause localized toxicity to the ciliary body epithelium. This may offer an alternate treatment of eyes in humans with end-stage blind, painful glaucoma.

References