Ultrastructural Changes in Rabbit Ciliary Body after Extraocular Mitomycin C

Rebecca S. Heaps, John R. Nordlund, Federico Gonzalez-Fernandez, Jan A. Redick, and Brian P. Conway

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 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 were vacuoles and expansion of intracellular and intercellular spaces. Plasma membrane infoldings were disrupted, and the apical membrane was thinned.


Mitomycin C (MMC), an antibiotic isolated from Streptomyces caespiotis 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 mytomycin 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 mitomycin 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; arrow, 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 glycocalyxlike 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 epithelial cells were disrupted. The glycocalyxlike apical membrane was thinned and intercellular spaces (Fig. 1).

The lowered intraocular pressures found after MMC trabeculectomy may be a result of hyposecretion of aqueous humor,\textsuperscript{3,7} overfiltration,\textsuperscript{2,6} or a combination of the two. Potential cytodestructive properties of MMC have been postulated. Letchinger et al.\textsuperscript{8} showed an intrinsic intraocular pressure-lowering effect of MMC in the rabbit model. Wilton et al.\textsuperscript{7} found that the intraocular pressure decreased with subconjunctival deposits of MMC in rabbits but not with topically applied MMC. Mietz and colleagues\textsuperscript{4} reported 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.\textsuperscript{9} showed similar transmission electron microscopic changes after MMC trabeculectomy in human ciliary body epithelium after death. In contrast, Hollo and Suveges\textsuperscript{5} found no morphologic changes in the ciliary epithelium of rabbits after exposure to MMC.

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