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The pharmacokinetics and effects of diltiazem in rabbits

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PURPOSE. *To investigate the effect of diltiazem on wound healing after the creation of conjunctival flaps in rabbit eyes. Also, to investigate the pharmacokinetics of diltiazem in rabbits after subconjunctival and topical administration.*
METHODS. *For the histopathological study, a limbal-based flap was prepared and diltiazem was injected subconjunctivally for five days after the surgery. The rabbits were euthanised 20 days after surgery. The effectiveness of diltiazem on wound healing was evaluated by histopathological examination and measurement of the thickness of subconjunctival fibrous tissue. For the pharmacokinetic study, diltiazem was applied topically or injected subconjunctivally. Aqueous paracenteses were performed 0.5, 1, 2, 4 hours thereafter.*
RESULTS. *The histopathological study found no difference in thickness of the subconjunctival fibrous tissue in control and diltiazem-treated eyes. No significant toxicity was observed in eyes treated with diltiazem. The peak aqueous concentration was 3.8 ± 0.4 µg/ml after topical application and 15.3 ± 1.1 µg/ml after subconjunctival injection. The peak aqueous concentration was achieved 1/2 hours after administration in both cases.*
CONCLUSIONS. *Diltiazem did not appear to affect wound healing at the dose tested. Topical and subconjunctival diltiazem successfully penetrated the aqueous humor of rabbit eyes. (Eur J Ophthalmol 2000; 10: 46-50)*

KEY WORDS: *Glaucoma, Diltiazem, Histopathology, High-performance liquid chromatography*

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INTRODUCTION

Glaucoma infiltration surgery fails primarily because of the wound healing process and scarring in the surgical area (1-3). To inhibit this, several agents have been studied experimentally *in vivo* and *in vitro* (4-10). Antimetabolites with antifibroplastic proliferative activity have been used to modify the wound healing response and increase the surgical success rate (11-13). Studies continue with other agents that safely and effectively inhibit fibroblast proliferation.

Calcium channel blockers have widespread physiological and pharmacological actions (14-15). The direct effect of topical calcium channel blockers on intraocular pressure has been studied in animals and humans,with variable results (16-18). Besides their effect on intraocular pressure, they inhibit the incorporation of proline into extracellular matrix protein by fibroblasts *in vitro* and prevent postoperative abdominal adhesions in animal models (19-23).

Diltiazem is one of this group of drugs (14-15). We studied the effect of this calcium channel blocker on

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wound healing after the creation of conjunctival flaps in rabbit eyes. We also studied the concentrations of the drug after topical application and subconjunctival injection to clarify the pharmacokinetics in rabbit eyes.

MATERIALS AND METHODS

Albino rabbits weighing between 2-3 kg were used. All the animal experiments were conducted in accordance with the ARVO resolution on the use of animals in research. Anesthesia was achieved with intramuscular injection of ketamine hydrochloride (25 mg/kg) and xylacine hydrochloride (5 mg/kg). Solutions of diltiazem hydrochloride were prepared in saline solution.

Histopathological study

For this study we used 10 albino rabbits. A lid speculum was placed and the conjunctiva was superiorly incised about 8 mm posterior from the limbus; a limbal-based conjunctival flap was prepared.

The conjunctiva was closed with an 8/0 polyglactin-running suture. Subconjunctival injections of 0.5 ml of diltiazem (10 mg/ml) were administered daily 180° from the flap site postoperatively under topical anesthesia for five days in the treated group (10 eyes). Ten control eyes received 0.5 ml of saline. Rabbits were examined twice a week for ulceration and surface defects. Polymycin ophthalmic ointment was instilled at the end of the procedure. No other postoperative medications were used. The animals were killed with intravenous pentobarbital sodium 20 days after surgery and the eyes were carefully removed to leave the superior conjunctiva relatively undisturbed. After enucleation, the eyes were immediately fixed in 10% neutral buffered formalin. Samples of surgical sites were prepared and embedded in paraffin. Five µm sections for light microscopy were stained with Masson trichrome and hematoxylin-eosin. The thickness of the subconjunctival fibrous tissue and cornea in all of the eyes was measured with a micrometer.

Diltiazem concentrations

We determined diltiazem concentrations by high-performance liquid chromatography (HPLC), using 16 albino rabbits to follow the concentration profile after topical application or subconjunctival injection. Diltiazem was dropped topically or injected subconjunctivally in both eyes of the rabbits, divided into two groups. The first group of rabbits received one 40 µl drop (10 mg/ml) onto the cornea of one eye and the lids were closed and opened for 30 seconds to simulate blinking and to ensure drug distribution. The second group received 0.5 ml diltiazem (10 mg/ml) by subconjunctival injection after topical anesthesia with 0.4 % oxybuprocaine hydrochloride. Rabbits were killed using 50 mg/kg of intravenous pentobarbital sodium at each of the following the intervals, 0.5, 1, 2, 4 hours after drug administration. Eyes were rinsed thoroughly with 0.9% sodium chloride solution and wiped dry with a cellulose sponge. Aqueous paracenteses were performed with a 25-gauge needle on a tuberculin syringe. Four eyes of two rabbits were used at each time and each drug concentration. Student's t test was used for statistical analysis.

RESULTS

Histopathological study

Examination of all eyes by light microscopy revealed normal intraocular structures, including the cornea, lens, ciliary body and retina. No morphological changes were observed in the corneal endothelium and ciliary body epithelium in either the experimental or control eyes (Figs. 1, 2). The thickness of the subconjunctival fibrous tissue did not differ in control and diltiazem-treated eyes. At 20 days, the control group had a mean subconjunctival fibrous tissue thickness of 425±35 µm, compared to 400 ± 40 µm in the diltiazem group. This was not statistically significant. The mean thickness of the corneas in experimental eyes was also no different from control eyes (400 ± 32.5 and 410 ± 30 µm).

Diltiazem concentrations

Intraindividual and interindividual differences in diltiazem concentrations were almost identical. The mean concentrations of diltiazem in the aqueous at different intervals after topical administration is shown in Table I. The peak concentration was obtained at 1/2 hour, after which diltiazem was quickly cleared from

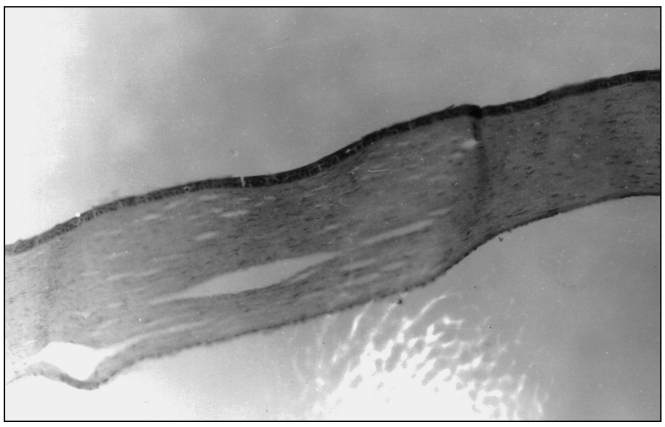

Fig. 1 - *Cornea with a normal histological appearance in a control rabbit (hematoxylin-eosin, x 20).*

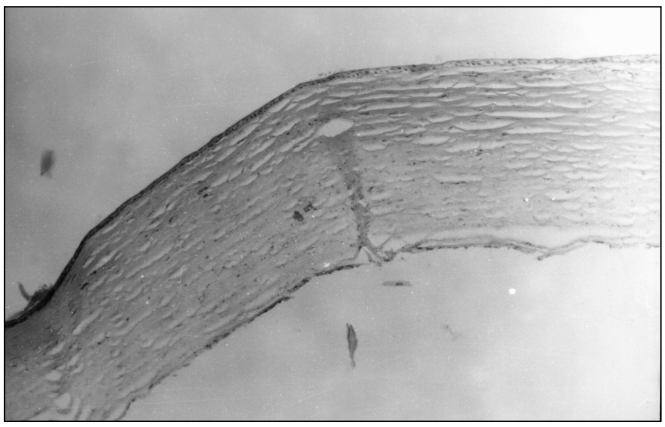

Fig. 2 - *Cornea of a diltiazem treated eye, with no histological difference from the control (hematoxylin-eosin, x 20).*

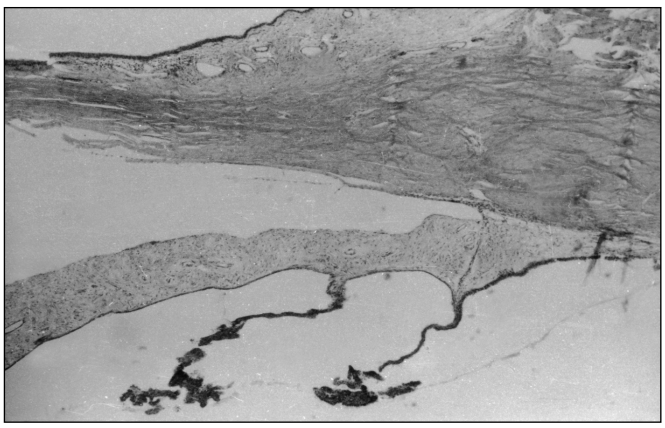

Fig. 3 - *Corpus ciliare with a normal histological appearance in a control rabbit (hematoxylin-eosin, x 20).*

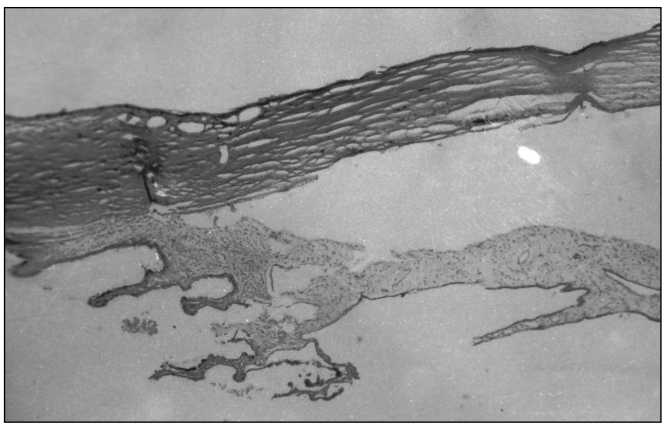

Fig. 4 - *Corpus ciliare of a diltiazem treated eye, with no histological difference from the control (hematoxylin-eosin, x 20).*

the anterior chamber, diappearing within 4 hours of administration. Table I also shows the mean concentration of diltiazem in the aqueous of the rabbit eyes at different intervals after subconjunctival injection. The concentration again peaked at 1/2 hour and diltiazem was nearly cleared from the anterior chamber within 4.

Diltiazem levels were higher after subconjunctival injection than topical application at 1/2 h, but the drug was cleared from the anterior chamber sooner after subconjunctival injection than topical instillation.

DISCUSSION

Diltiazem and verapamil are both calcium channel blockers (14, 15). There are few reports on the effects

TABLE I - AQUEOUS CONCENTRATION OF DILTIAZEM IN RELATION TO THE TIME AFTER APPLICATION OF TOPICAL DROPS OR SUBCONJUNCTIVAL INJECTIONS (mean ± standard error)

	Aqueous concentration (µg/ml)	
Time (h)	**Topical application**	**Subconjunctival injection**
0.5	3.8 ± 0.4	15.3 ± 1.1
1.0	2.2 ± 0.5	1.58 ± 0.5
2.0	1.2 ± 0.4	0.50 ± 0.2
4.0	0.28 ± 0.1	0

of subconjunctival or topical verapamil on filtering blebs in rabbits (22-23). Gupta (22) showed that verapamil reduced scar formation at the sclerostomy site; verapamil was non-toxic at the dose administered in their model. Siegfried (23) demonstrated that human fibroblast proliferation was inhibited by incubation with verapamil and blebs in the treated eyes appeared more avascular than control. In the present study, the only one done with diltiazem, we did not find the drug helped wound healing after the creation of conjunctival flaps in rabbit eyes.

The adjunctive use of antimetabolites has revolutionized glaucoma surgery in the past decade (8-13) but their use has been accompanied by a variety of complications that may limit their application in primary trabeculectomies (24-28). In our study none of the specimens showed evidence of scleral calcification, thinning, ulceration, or necrosis. Likewise, there was no evidence of corneal ulceration, corneal stromal scarring or perforation. Other ocular structures also appeared histologically normal. Diltiazem appears to be non-toxic at the dose administered in this model.

There is one report on the pharmacokinetics of verapamil. Ettl (29) showed that topically administered verapamil produced 200 times higher peak drug levels in aqueous humour and negligible serum levels, compared to systemic administration. We determined the pharmacokinetics of diltiazem after topical or subconjunctival delivery in healthy rabbits. The aqueous concentration of diltiazem peaked at 1/2 hour with both routes. Maximum aqueous concentrations were approximately four times greater after subconjunctival than topical administration. Both administration routes resulted in successful penetration of diltiazem into the aqueous humour, and the drug disappeared rapidly from the anterior chamber.

This study indicates that postoperative subconjunctival diltiazem did not appear to affect wound healing at the dose tested. Further studies in non-human primates are needed to clarify the dose-response patterns and efficacy of diltiazem.

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REFERENCES

1. Skuta GL, Parrish PK. Wound healing in glaucoma filtering surgery. Surv Ophthalmol l987; 32: 149-70.
2. Addicks EM, Quigley HA, Green W, Robin AL. Histologic characterestics of filterings blebs in glaucomatous eyes. Arch Ophthalmol 1983; 101: 705-98.
3. Joseph JP, Miller MH, Hitchings RA. Wound healing as a barrier to successful filtration surgery. Eye 1988; 2 (suppl): S113-23.
4. Lee DA, Leong KW, Panek WC, Eng CT, Glaskow BJ. The use of bioerodible polymers and 5-fluorouracil in glaucoma filtration surgery. Invest Ophthalmol Vis Sci 1988; 29: 1692-97.
5. Kay JS, Fryczkowski AW, Litin BS, Chvapil M, Jones MA, Herschler J. Delivery of antifibroblast agents as adjuncts to filtration surgery, II: delivery of 5-fluorouracil and bleomycin in collagen implants: a pilot study in the rabbit. Ophthalmic Surg 1986; 17: 796-801.
6. Fourman S, Vaid K. Effects of tissue plasminogen activator on glaucoma filter blebs in rabbits. Ophthalmic Surg 1989; 20: 663-7.
7. Miller MH, Grierson I, Unger WG, Hitchings RA. The effect of topical dexamethasone and preoperative beta irradiation on a model of glaucoma fistulizing surgery in the rabbit. Ophthalmic Surg 1990; 21: 44-54.
8. McGuigan LJB, Cook DJ, Yablonski ME. Dexamethasone, D-Penicillamine and glaucoma filter surgery in rabbits. Invest Ophthalmol Vis Sci 1986; 27: 1755-57.
9. Lee DA, Lee TC, Cortes AE, Kitada S. Effects of mithramycin, mitomycin, and bleomycin on human subconjunctival fibroblast attachment and proliferation. Invest Ophthalmol Vis Sci 1990; 21: 2136-44.
10. Yamamoto T, Varani J, Soong HK, Lichter PR. Effects of 5-fluorouracil and mitomycin-C on cultured rabbit subconjunctival fibroblast. Ophthalmology 1990; 97: 1204-10.
11. Heuer DK, Parrish RK II, Gressel MG, et al. 5-Fluorouracil and glaucoma filtering surgery: III. Intermediate follow-up of a pilot study. Ophthalmology 1986; 93: 1537-46.
12. Kitazawa Y, Kawase K, Matsushita H, Minobe M. Trabeulectomy with mitomycin. A comparative study with 5-fluorouracil. Arch Ophthalmol 1991; 109: 1693-8.
13. Skuta GL, Beeson CC, Higginbotham EJ, et al. Intra-

operative versus postoperative mitomycin in high-risk glaucoma filtering surgery. Ophthalmology 1992; 99: 438-44.
14. Piepho RW. The calcium antagonist. Mechanism of action and pharmacologieal effects. Drug Ther Bull 1983; 13: 68-84.
15. Braunwald E. Mechanisms of calcium-channel-blocking agents. N Engl J Med 1982; 307: 1618-27.
16. Payne LJ, Slagle TM, Cheeks LT, Green K. Effect of calcium channel blockers on intraocular pressure. Ophthalmic Res 1990; 22: 337-41.
17. Netland PA, Chaturvedi N, Dreyer EB. Calcium channel blockers in the management of low tension and open-angle glaucoma. Am J Ophthalmol 1993; 115: 608-13.
18. Abelson MB, Gilbert CM, Smith LM. Sustained reduction of intraocular pressure in humans with the calcium channel blocker verapamil. Am J Ophthalmol 1988; 105: 155-59.
19. Steinletner A, Lambert H, Kazensky C, Sanchez I, Sueldo C. Reduction of primary postoperative adhesion formation under calcium channel blockade in the rabbit. J Surg Res 1990; 48: 42-5.
20. Steinletner A, Lambert H, Montoro L, Kelly E, Swanson J, Sueldo C. The use of calcium channel blockade for the prevention of postoperative adhesion formation. Fertil Steril 1988; 50: 819-21.
21. Steinletner A, Lambert H, Montoro L, Swanson J, Sueldo C. Use of diltiazem for preventing postoperative adhesions. J Reprod Med 1988; 33: 891-4.
22. Gupta B, Agarwala A, Moy JJ, Edward DP. The effects of subconjunctival verapamil on filtering blebs in rabbits. Ophthalmic Surg 1996; 27: 378-83.
23. Siegfried C, Mc Daniel L, Karalekas D, Rosenberg I, Ruderman J, Krupin T. The effect of topical verapamil on filtering bleb survival in rabbits. Invest Ophthalmol Vis Sci 1994; 35 (suppl): S1899.
24. Knapp A, Hever DK, Stern GA, Driebe WT Jr. Serious corneal complications of glaucoma filtering surgery with postoperative 5-fluorouracil. Am J Ophthalmol 1987; 103: 183-87.
25. Franks WA, Hitchings RA. Complications of 5-fluorouracil after trabeculectomy. Eye 1991; 5: 385-9.
26. Ticho U, Ophir A. Late complications after glaucoma filtering surgery with adjunctive 5-fluorouracil. Am J Ophthalmol 1993; 115: 506-10.
27. Zacharia PT, Deppermann SR, Schuman JS. Oculer hypotony after trabeculectomy with mitomycin C. Am J Ophthalmol 1993; 116: 314-26.
28. Rubinfeld RS, Pfister RR, Stein RM, et al. Serious complications of topical mitomycin-C after pterygium surgery. Ophthalmology 1992; 99: 1647-54.
29. Ettl A, Dexter A, Hoffmann U. Calcium channel blockers in the management of low tension and open angle glaucoma (Letter). Am J Ophthalmol 1993; 116: 778.