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It has been suggested that corticosteroids plus the effective antibiotics are more beneficial than antibiotics alone in reducing inflammation in infectious keratitis. Topical and subconjunctival corticosteroids as adjuncts to gentamicin therapy were evaluated by microbiological assay and clinical parameters in experimentally induced Pseudomonas keratitis in rabbits. Various strengths of dexamethasone given concurrently or delayed in conjunction with gentamicin therapy were studied. There was no statistically significant difference between combinations and the antibiotics alone in this experimental model.

Over the past few years, ocular infections by Pseudomonas aeruginosa have been increasing, especially in patients hospitalized with underlying debilitating ocular and systemic diseases. Uncontrolled, these infections can lead to a rapid loss of vision and destruction of the eye. Although new antibiotics are bactericidal, frequently it is the secondary inflammatory process which causes destruction of the cornea. The use of corticosteroids in corneal infections has been controversial. It has been suggested that corticosteroids plus the effective antibiotics are more beneficial than the antibiotics alone in reducing the disease process. Aronson and Moore have recommended combined high-dosage topical corticosteroid therapy in active Pseudomonas keratitis. Some have questioned whether high-dose steroids would enhance or prolong Pseudomonas viability. The rationale for the addition of steroids to the appropriate antibiotic in the treatment of infectious keratitis is to reduce the severity of the inflammatory response, thus reducing corneal scarring. Newmark and Ellison have suggested that weaker steroid concentrations are better be-

cause they promote anti-inflammatory effects without suppressing local host resistance. The purpose of this study was to evaluate the effects of gentamicin and corticosteroids in combination, in the treatment of Pseudomonas keratitis in rabbits. Similar studies have been done in the past but have not been approached microbiologically. The parameters used as end points in this study were (1) the microbiological assay and (2) the clinical assay of keratitis.

Materials and methods

Animals. Female New Zealand white rabbits (weighing 2 kg.), free from ocular lesions and disease, were used in the study.

Pseudomonas strain. P. aeruginosa strain, pyro-
cine type 6, from a lyophilized pool was prepared with sterile saline on a blood agar plate for each experiment. This strain was sensitive to 10 μg gentamicin sulfate disks. A 24 hr. growth was used for inoculation. This species was originally isolated from a clinical case of Pseudomonas keratitis.

Drugs. Commercially available gentamicin sulfate (Garamycin; Schering Corp., Bloomfield, N. J.) and dexamethasone disodium phosphate (Decadron; Merck, Sharp & Dohme, West Point, Pa.) were used topically and subconjunctivally. Two drops (0.12 cc.) of gentamicin sulfate, 3.0 mg./cc., were given four times a day; a subconjunctival injection of 10 mg. of gentamicin in 0.25 cc. was also administered in the evening. Dexamethasone was given in a similar manner, 2 drops (0.12 cc.) times four times a day. In certain rabbits, subconjunctival injections of 1 mg. of dexamethasone in 0.25 cc. were given in addition to the topical steroid. In eyes receiving more than one type of agent, subconjunctival injections were given in different quadrants, and there was a delay of 5 min. between different drops so as to minimize dilution factors. The control solution for dexamethasone was the vehicle supplied by Merck, Sharp & Dohme. The control for antibiotic was normal saline.

Experimental design. Each cornea (in both eyes) was inoculated centrally with a 30-gauge microsyringe (Hamilton Co., Reno, Nev.) containing 0.03 ml. of a suspension of approximately 100 viable P. aeruginosa organisms intrastromally. This suspension was prepared from a 24 hr. growth of the above strain of Pseudomonas at 37° C. on blood agar plates. Dilutions were done with physiological saline to produce a standardized suspension to 95% transmission at a wavelength of 540 nm. against a clear blank in a Coleman spectrophotometer (Coleman Instruments, Oak Brook, Ill.). Injections were made under a topical proparacaine hydrochloride (Ophthaine, Alcaine) anesthetic in the central corneas of all rabbit eyes with the use of an operating microscope. This dosage, based on preliminary study, was found to
Fig. 1. Representative course of disease in antibiotic-treated eyes vs. saline-treated control eyes.

Table I. Treatment groups

<table>
<thead>
<tr>
<th>No. of rabbits</th>
<th>Treatment*</th>
<th>OD (control)</th>
<th>OS (combinations)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (antibiotic alone)</td>
<td>10</td>
<td>Normal saline top. q.i.d. &amp; subconj. q.h.s.</td>
<td>Gentamicin top. q.i.d. + gentamicin, 10 mg. subconj. q.h.s.</td>
</tr>
<tr>
<td>Group 2 (steroid alone)</td>
<td>5</td>
<td>Normal saline subconj. &amp; subconj. q.h.s.</td>
<td>0.1% dexamethasone top. q.i.d. + dexamethasone 1 mg. subconj. q.h.s.; no antibiotic</td>
</tr>
<tr>
<td>Group 3 (medium dose steroids)</td>
<td>5</td>
<td>Gentamicin top. q.i.d. + gentamicin 10 mg. subconj. q.h.s.</td>
<td>0.1% dexamethasone top. q.i.d. + gentamicin top. q.i.d. &amp; gentamicin 10 mg. subconj. q.h.s.</td>
</tr>
<tr>
<td>Group 4 (medium dose steroids delayed 24 hr.)</td>
<td>5</td>
<td>Gentamicin top. q.i.d. + gentamicin 10 mg. subconj. q.h.s.</td>
<td>0.1% dexamethasone top. q.i.d., delayed 24 hr. + gentamicin top. &amp; subconj.</td>
</tr>
<tr>
<td>Group 5 (lower dose steroids)</td>
<td>10</td>
<td>Gentamicin top. q.i.d. + gentamicin 10 mg. subconj. q.h.s.</td>
<td>0.001% dexamethasone top. q.i.d. + gentamicin top. &amp; subconj.</td>
</tr>
<tr>
<td>Group 6 (higher dose steroids)</td>
<td>7</td>
<td>Gentamicin top. q.i.d. + gentamicin 10 mg. subconj. q.h.s.</td>
<td>0.1% dexamethasone top. &amp; subconj. + gentamicin top. &amp; subconj.</td>
</tr>
</tbody>
</table>

*Treatment began 4 hr. after inoculation and continued for 7 days.

give an effective but not overwhelming keratitis when inoculated into the rabbit cornea. The animals were divided into six groups (Table I). Intracorneal injection was chosen as the method of injection to maintain as constant as possible the number of organisms inoculated.

Treatment was begun 4 hr. after inoculation. All the animal eyes except those in control groups 1 and 2 were treated with gentamicin topically and subconjunctivally. Group 1 was an infection control group receiving saline in the right eye and gentamicin alone in the left eye. In group 2 saline was compared to steroid alone. In groups 3 to 6, the right eye of all animals received gentamicin only, and the left eye received dexamethasone in varying dosages in addition to the gentamicin; thus the right eye served as the control. Treatment was carried out for 7 days. Each day the eyes were clinically scored, each eye receiving scores of 0 to +4, on the basis of the area of corneal infiltrate, as previously described.

On day 8, the animals were sacrificed. Corneas and cul-de-sacs were irrigated with sterile, normal saline. Under sterile conditions, the corneas were removed with a 10 mm. trephine, minced, and ground in a test tube mechanical grinder with 1 cc of trypticase soy broth and plated directly on blood agar plates. At 24 and 48 hr., the colonies were counted.

Results. The experimental design was estab-
trol eye. Five rabbits receiving the steroid-anti-
organisms recovered and the clinical score. The
there was a correlation between the number of
organisms recovered (group 1, >100, +4), 3 +1
2 >100 +4  4 0
3 >100 +4  4 +4
5 2 +4  1 +4
6 >100 +4  0 0
7 2 +4  0 +2
8 15 +4  26 +4
9 40 +4  0 0
10 >100 +4  0 0
Average 71 +4  3.4 +1

Gentamicin was used as the effective antibiotic
dose in these studies. Meldrum and limits 8
suggested that the optimal dose could be
0.3 mg./animal for 5 days. There have been no
well-controlled studies in humans to determine if the
addition of steroid is more beneficial than the effective antibiotic
dose. 5

Discussion. To the best of our knowledge,
there have been no well-controlled studies in
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designed to correspond to the human disease as closely as possible.

Though this pilot investigation was in animals, the notion that acute *Pseudomonas* keratitis is better treated with combined steroid antibiotic therapy cannot be supported by this present study. The therapeutic benefit of addition of steroids to antibiotics on human cases of *Pseudomonas* corneal ulcer will depend on the results of well-controlled studies in the future.

Our conclusions may be summarized as follows. (1) The use of steroids in combination with gentamicin coverage did not significantly alter the course of keratitis as measured by microbiological assay or clinical study in this experimental protocol. (2) This was true whether steroid therapy was started early or delayed in either higher or lower strengths. (3) There was a correlation between the severity of keratitis and the number of organisms recovered at the time of assay. (4) Clinically inactive eyes can harbor viable *Pseudomonas* after 1 week of therapy.

From the Department of Ophthalmology, Washington University Medical Center, St. Louis, Mo. This study was supported by research grants from the National Society for the Prevention of Blindness, Inc. and Fausek Ophthalmology Foundation Fund. Read before the Association for Research in Vision and Ophthalmology, Sarasota, Florida, May, 1975. Submitted for publication, May 18, 1976. Reprint requests: George M. Bohigian, M.D., Washington University School of Medicine, Department of Ophthalmology, 660 South Euclid Ave., St. Louis, Mo. 63110.

Key words: *Pseudomonas aeruginosa*, keratitis, antibiotics, corticosteroids, gentamicin, subconjunctival antibiotics, subconjunctival steroids, cornea.

REFERENCES


A further examination of short-term corneal storage. J. S. CHAPMAN-SMITH.

Short-term corneal storage at a temperature of 4° C. was assessed with human cadaver eyes. Aqueous was replaced by air in each of 11 pairs of eyes, stored for periods of up to 96 hours post-mortem. The influence of air on the endothelial layer was measured in terms of corneal thickness, trypan blue staining, and scanning electron microscopy. Air-filled eyes developed less corneal edema over the storage period. No significant differences were found between the endothelia of test and control eyes. Degenerative changes in endothelium were documented photographically.

Donor corneae have been shown to remain viable after storage in McCarey-Kaufman medium for 3 to 4 days, after storage in recipient serum for up to 100 hours, and in liquid paraffin at 4° C. for 3 weeks. The longest period of successful storage is 422 days, in liquid nitrogen. However, the commonest, simplest technique is the early removal of a donor eye from a cadaver and storage in a sterile container at 4° C.

Some ophthalmologists are critical of leaving the aequous in contact with the endothelium in the stored eye. McCarey and Kaufman describe how the aequous contains "products from metabolic wastes and necrosis of tissue." A recent report suggests this is a major reason for storing the cornea separately from the eyeball. In 1965, Klen and associates, after studying cattle eyes stored at 4° C, concluded that corneal transparency reflected corneal viability in their stored cornea and that a number of agents, including air, which they had placed in the anterior chamber, prolonged corneal transparency.

This study tests the hypothesis that intracameral air prolongs the life of corneal endothelium stored at 4° C. and aims to document the endothelial changes occurring, with time, in the stored control eyes.

Methods. Eleven pairs of human eyes which appeared clinically healthy were enucleated as soon after death as practical. A 26-gauge needle on a tuberculin syringe was introduced through the limbal region of the test eye. Aqueous (0.2 ml.) was withdrawn and replaced by a similar volume of air. The second eye of each pair served as a control. Each pair of eyes was stored in the same sterile moist chamber, cornea uppermost, at a constant temperature of 4° C. A further cornea was obtained at operation from a 47-year-old woman with keratoconus (Case Y).

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