Nebcin® in the treatment of experimental Pseudomonas keratitis

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Pseudomonas aeruginosa is one of the most virulent and frequently encountered infectious agents affecting the cornea (Allen and Mangiaracine, 1964). Unhappily, its destructive effect on ocular tissues cannot always be prevented by the antibiotics currently available. The best anti-Pseudomonas drugs are colistin, polymyxin B, and gentamicin (Burns, 1969; Gordon, 1970), but Pseudomonas strain-resistance to gentamicin is increasing (Morel, Fremy, and Monacroq, 1972), and all three drugs can produce unfortunate side-effects, such as conjunctival scarring, when injected subconjunctivally.

Nebramycin is an aminoglycoside complex that was isolated from a culture of Streptomyces tenebrarius (Higgens and Kastner, 1967). In suitable media, this species produces a group of eight chemically-related antibiotic factors, of which tobramycin (Factor 6) has been found to have the highest specific effect on P. aeruginosa (Wick and Welles, 1967). Its level of nephrotoxicity is slightly less than that of gentamicin, but it is just as ototoxic as gentamicin and kanamycin (Wick and Welles, 1967; Eli Lilly & Co., 1974); and there is evidence that it is active in vitro and in vivo against some gentamicin-resistant strains (Smolin, Okumoto, and Wilson, 1974).

When tobramycins is injected subconjunctivally in concentrations bactericidal to P. aeruginosa (Purnell and McPherson, 1974), it rapidly penetrates the eye. And since it is now available as an injectable solution (Nebcin®*), we decided to test both its efficacy and its toxicity when used to treat Pseudomonas-infected rabbit eyes, and to compare these effects with those of gentamicin.

*Nebcin® (tobramycin sulphate), Eli Lilly & Co., Indianapolis, Ind.

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Material and methods

A. IN VITRO TESTING

Minimum inhibitory concentrations (MIC) of Nebcin® and gentamicin

To determine with precision the inhibitory effects of Nebcin® and gentamicin on P. aeruginosa in vitro, we tested solutions of both drugs (40 mg/ml) by the quantitative tube-dilution method. To this end we prepared nine serial two-fold dilutions of both solutions. In each series of nine tubes, the highest final concentration was 5 µg/ml and the lowest 0.019 µg/ml. Each series was prepared in triplicate.

The test culture was a 24-hr broth culture of P. aeruginosa (El Salvador strain No. 4, pyocine type 6) incubated at 37°C. We diluted this culture a thousand fold, placed 1 ml of the final suspension in each of the tubes containing 1 ml of the various concentrations of the antibiotics, and incubated the tubes at 37°C for 24 hr. In each series of tubes, the lowest antibiotic concentration that inhibited the growth of Pseudomonas was the MIC of that antibiotic. Samples of the tubes in which there was no apparent growth of organisms were then cultured to determine the minimum bactericidal concentration of each antibiotic.

B. IN VIVO TESTING

1. Ocular toxicity

Our experimental animals were nine New Zealand white (NZW) rabbits weighing 2–3 kg each. We injected both eyes of three groups of three rabbits each subconjunctivally with 0.2 ml gentamicin (40 mg/ml), 0.2 ml of Nebcin® (40 mg/ml), and 0.2 ml saline solution, respectively, twice a day for 3 days.

We examined the eyes daily for a 6-day period and graded the degree of conjunctival exudate, oedema, hyperaemia, haemorrhages, and scarring, and of any detectable corneal pathology. The animals were killed on day 11. Specimens consisting of the entire upper lid, conjunctiva, and fornix were excised and fixed in neutral formalin. After embedding and sectioning the fixed tissues, we stained the deparaffinized sections (5 µm) with haematoxylin and eosin and examined them for histological evidence of the drugs' toxic effects on the eye.
length with sufficient pressure to penetrate the superficial corneal stroma. (After each inoculation the curette was cleaned mechanically with a sterile cotton-tipped applicator.)

The eyes were examined 24 hr after inoculation and the corneal lesions graded from 0 to +5 according to their size and density as follows:

- **0** = No effect
- **+1** = Up to 33 per cent of the cornea affected
- **+2** = 34-66 per cent of the cornea affected
- **+3** = 67 per cent or more of the cornea affected

If the corneal opacification was nebular, we added nothing to the score; if it was macular, we added 1; and if it was leucomatous, we added 2. The highest possible score was thus +5.

2. **Clinical efficacy and culture study**

We used 24 male NZW rabbits weighing 2-3 kg each and with initially normal anterior ocular segments. We induced *Pseudomonas* keratitis in both eyes of each rabbit by the Cignetti method (Smolin, Okumoto, and Wilson, 1973), as follows:

After anesthetizing each eye by the instillation of proparacaine HCl (Ophthaine®), we filled the cup of a toothed chalazion curette (No. SP 40315 Storz Surgical Instruments, St Louis, Missouri) with a confluent growth of a 24-hr blood-agar culture of the same El Salvador strain of *Ps. aeruginosa* used in our in vitro study. We then made a single central abrasion 8 mm in
After grading the lesions, we swabbed the corneas of each rabbit with a moistened, sterile, cotton-tipped applicator, streaked the swabs over blood-agar culture plates, incubated the plates for 24 hr at 37°C, and counted and recorded the number of colonies on each plate.

The animals were divided into three clinically similar groups. Twice a day for 3 days, each group received subconjunctival injections 0.2 ml of one of the following solutions: saline solution (control), Nebcin® (40 mg/ml), and gentamicin (40 mg/ml). Each morning before treatment, two of us graded the lesions. On day 4 we killed the animals and excised their eyes. We ground each cornea separately in a mortar with sterile sand and nutrient broth until we had a homogenous suspension. This entire corneal suspension was cultured on blood agar and incubated at 37°C for 48 hr, and the number of colonies on each plate was then counted and recorded.

The organisms recovered from each of the cultured corneas of each rabbit were tested by the tube-dilution method against the same antibiotic used to treat the rabbit. The antibiotic sensitivity of the organisms from the cultured corneas was then compared with their initial sensitivity.

On six rabbits we calculated the approximate number of organisms delivered to the rabbit eye by the cup of the curette by measuring, first, the number required to fill the cup, and second, the number left in the cup after the cornea had been abraded. The difference between the full cup and what was left after the abrasion (that is, the number delivered to the cornea) was approximately 2 × 10⁶.

Results

A. IN VITRO TESTING

MIC of Nebcin® and gentamicin

For both antibiotics tested, the same minimum concentrations were bactericidal as well as inhibitory, but in measuring the MIC, Nebcin® appeared to be more active than gentamicin by at least one tube dilution; its MIC was 0.62 μg/ml and that of gentamicin 1.25 μg/ml.

B. IN VIVO TESTING

1. Ocular toxicity

In the nine rabbits tested for the drugs’ conjunctival and corneal toxicity, there was no clinical difference between the toxic effects of Nebcin® and gentamicin. Both drugs produced moderate inflammation with conjunctival oedema, hyperaemia and subconjunctival haemorrhages. These changes subsided fairly rapidly so that in 4 to 6 days the eyes were again clinically normal. Neither drug produced any corneal lesions.

Histological sections of conjunctival specimens from all three groups showed vascular dilatation, neovascularization, and cellular infiltration. The control group (treated with saline solution) showed fewer pathological alterations than the antibiotic-treated groups. The corneas of both the Nebcin®- and gentamicin-treated groups showed a polymorphonuclear cellular infiltration with some necrosis and fibroblastic infiltration.

2. (a) Clinical efficacy

We averaged the clinical scores for each eye for the first, second, and third days of treatment, and on the day when the rabbits were killed (Table I). When we applied the Wilcoxon signed-rank test, the saline-solution-treated (control) group showed a progressive and significant worsening of the corneal lesions during the 3 days of treatment (P < 0.01). In the gentamicin-treated group there was a significant worsening of the lesions between days 2 and 3 (P < 0.5); but in the Nebcin®-treated group there were no statistically significant changes in the lesions from the beginning of therapy.

When we applied the median test, the Nebcin® group showed a statistically significant difference on each of the 3 days of treatment when compared with the saline-solution group (Table I); the gentamicin group showed a significant difference only on day 2 when compared with the saline-solution group (P < 0.01); and the Nebcin® group was significantly better than the gentamicin group only on day 3 (P < 0.05).

(b) Colony counts on culture plates

Table II presents the cultural results. Before treatment Student’s t test showed that there were significantly more colonies on the plates to be treated with gentamicin and Nebcin® than there were on the control group plates (P < 0.05).

After treatment the t test showed significantly fewer colonies on the plates from the Nebcin®- and gentamicin-treated groups than on those from the saline-solution-treated group (P < 0.01). There were in fact no colonies at all on the plates from the Nebcin®-treated group, which was significantly better than the gentamicin-treated group (P < 0.05). The antibiotic-sensitivity tests run on the Pseudomonas organisms recovered from the cultured corneas showed the same MIC as the tests run before the experimental infection had been induced.

It is important to emphasize that both antibiotics were active against Pseudomonas infection but that only in the Nebcin®-treated group were all of the corneas sterilized.

Discussion

Pseudomonas aeruginosa, an opportunistic pathogen, appears to be causing a steadily increasing number of ocular infections in immunologically-compromised hosts (Fromer and L’Esperance, 1971). Since the
organism is highly destructive to the eye and insensitive to most antibiotics, it is not surprising that many ophthalmologists have tried to find better agents to cope with it (Burns, 1969; Fromer and L’Esperance, 1971; Gordon, 1970; Smolin and others, 1974).

When we compared Nebcin® (a newly available antibiotic) with gentamicin (to date an effective anti-*Pseudomonas* drug), we found that the toxic effect of Nebcin® on the conjunctival tissues was the same as that of gentamicin, but that Nebcin® had more effect on our particular strain of *P. aeruginosa*, both in vitro and in vivo, than gentamicin. In the groups of rabbits treated with saline solution or gentamicin, the corneas became significantly worse from day 3 until the animals were killed (P < 0.5), but in the Nebcin®-treated group there were no statistically significant corneal changes from the first day of therapy. In culture studies, moreover, we recovered a few organisms from the gentamicin-treated rabbits after 3 days of treatment but none at all from the Nebcin®-treated rabbits. When the antibiotic sensitivity of the organisms persisting in the gentamicin-treated rabbits was tested in vitro, it was found to be the same as the sensitivity of the organisms tested before the experimental infection was induced.

**Summary**

When the ocular toxicity and the in vivo and in vitro effects of gentamicin, Nebcin®, and saline...
Table II  Number of colonies recovered from experimental Pseudomonas keratitis before and after treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rabbit no.</th>
<th>Before treatment*</th>
<th>After treatment**</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Right</td>
<td>Left</td>
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<tr>
<td>Saline solution</td>
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<td>200</td>
<td>51</td>
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<td></td>
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<td>Group totals</td>
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<td>Gentamicin</td>
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<td>Group totals</td>
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</table>

* Entire corneal surface swabbed  ** Whole cornea excised, ground, and cultured  *** Too numerous to count

Treatment of experimental Pseudomonas keratitis

Solution were compared in experimentally induced Pseudomonas keratitis in rabbits, both antibiotics showed the same toxicity for the rabbits' conjunctival tissues. But Nebcin® showed better in vitro and in vivo results than gentamicin, and the clinical effect was confirmed by culture study: significant numbers of organisms were recovered from the corneas of the gentamicin-treated rabbits but none from the corneas of the Nebcin®-treated rabbits.

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