Experimental *Pseudomonas* keratitis in guinea-pigs: therapy of moderately severe infections

STARKEY D. DAVIS, LARRIE D. SARFF, AND ROBERT A. HYNDIUK

From the Departments of Pediatrics and Ophthalmology, Medical College of Wisconsin, Milwaukee Children's Hospital, and the Cornea-External Disease Unit, The Eye Institute, Milwaukee, Wisconsin, USA

SUMMARY We have previously shown that antibiotic therapy of experimental *Pseudomonas* keratitis was more effective in early moderate infections than in late severe infections. The purpose of this study was to determine the relative efficacies of various drugs, routes, and vehicles in the treatment of moderately severe infection. As in the late severe infections, the most consistently effective regimen was an aminoglycoside applied topically in solution. No synergistic or additive effect was observed with a combination of aminoglycoside given topically and a penicillin given intramuscularly. Topical therapy with antibiotic in ointment was less effective than topical therapy with antibiotic in solution.

We have developed a reproducible, quantitative model of experimental *Pseudomonas* keratitis in guinea-pigs. Animals are infected by intracorneal injection to ensure that all corneas receive the same inoculum. Within 24 hours all infected corneas show frank keratitis. Results of treatment are assessed objectively by determining the number of bacteria surviving in the cornea (Davis and Chandler, 1975; Davis *et al.*, 1977; Davis *et al.*, 1978).

Efficacy of treatment of experimental *Pseudomonas* keratitis in guinea-pigs was enhanced by beginning therapy sooner after infection rather than later when a more severe infection was present. Earlier therapy was presumably more effective in part because there were fewer bacteria in the cornea (Davis *et al.*, 1978).

At 16 hours after infection in this model the number of viable bacteria in the cornea has increased from a geometric mean of 10 to a geometric mean of 48 000. An abscess is beginning to form in the cornea. After 24 hours a geometric mean of 280 000 organisms are present and abscess formation is more advanced (unpublished observations). We believe that a patient with a corneal infection may well first seek medical attention at the 16-hour stage.

Here we present results of trials in which antibiotic therapy was begun 16 hours after infection. Our purpose was to determine whether the relative efficacy of antibiotics would change when infection was less severe.

Materials and methods

These have been previously reported in detail (Davis and Chandler, 1975; Davis *et al.*, 1977; Davis *et al.*, 1978). Briefly, male Hartley strain guinea-pigs weighing 300 to 350 g were infected intracorneally with about 10 viable *Pseudomonas aeruginosa* strain 107, 110, or 113. Antibiotic therapy was begun 16 hours later. At the end of the experiments the animals were killed and the corneas were excised and ground in broth. Numbers of viable bacteria were determined by quantitative subcultures. The results were analysed statistically by analysis of variance (Snedecor and Cochran, 1967). Data are presented as means and standard deviations of the numbers of viable bacteria in common logarithms (base 10). The mean of numbers expressed in common logarithms is equivalent to the geometric mean. Analysis of data in this way dampens the effect of single extreme values.

Antibiotics were obtained through the hospital pharmacy or directly from the pharmaceutical company. Parenteral formulations of aminoglycosides were diluted in sterile 0.15 M NaCl for

Address for reprints: Dr Starkey D. Davis, MD, Department of Pediatrics, Milwaukee Children's Hospital, 1700 West Wisconsin Avenue, Milwaukee, Wisconsin 53233, USA

Table 1 Therapy of moderately severe experimental *Pseudomonas* keratitis in guinea-pigs*

Treatment	Corneal colony counts		P
	Mean $\pm$ SD	(N)	
0.15 M NaCl	6.18 $\pm$ 0.43	(6)	
Tobramycin 20 mg/ml, topical	1.73 $\pm$ 0.47	(6)	} > 0.1 } < 0.005
Gentamicin 20 mg/ml, topical	1.73 $\pm$ 0.48	(6)	
Polymyxin B, neomycin, gramicidin ophthalmic solution	1.46 $\pm$ 0.42	(6)	
Polymyxin B, bacitracin, neomycin ophthalmic ointment	5.43 $\pm$ 0.26	(6)	
Ticarcillin 100 mg/kg IM every 90 min	3.91 $\pm$ 0.56	(6)	} < 0.05
Carbenicillin 200 mg/kg IM every 90 min	4.56 $\pm$ 0.54	(6)	
Tobramycin 20 mg/ml topical, and ticarcillin 100 mg/kg IM every 90 min	1.51 $\pm$ 0.87	(6)	

*Animals were infected intracorneally and treatment was begun 16 hours later. Topical treatment was given as 2 drops every 30 minutes. Ointment was applied every 2 hours. Treatment was given for 16 hours, and then all animals were killed.

Table 2 Therapy of moderately severe experimental *Pseudomonas* keratitis*

Treatment	Corneal colony counts		P
	Mean $\pm$ SD	(N)	
0.15 M NaCl	6.07 $\pm$ 0.89	(6)	
Tobramycin 20 mg/ml, topical	0.70 $\pm$ 0.78	(6)	} > 0.1 } < 0.005
Gentamicin 20 mg/ml, topical	0.17 $\pm$ 0.41	(6)	
Amikacin 20 mg/ml, topical	0.79 $\pm$ 0.88	(6)	
Polymyxin B, neomycin, gramicidin ophthalmic solution, topical	1.19 $\pm$ 1.99	(6)	
Polymyxin B, bacitracin, and neomycin ophthalmic ointment, topical	4.06 $\pm$ 1.50	(6)	
Ticarcillin 100 mg/kg IM	1.85 $\pm$ 1.33	(6)	
Ticarcillin 100 mg/kg IM, and tobramycin 20 mg/ml, topical	0.33 $\pm$ 0.52	(6)	

*Therapy was begun 16 hours after infection and was given continuously for 24 hours. Topical solutions were given 2 drops every 30 minutes. Ointment was applied topically every 2 hours. Ticarcillin was given IM every 90 minutes.

topical use. An ophthalmic solution of polymyxin B 5000 units/ml, neomycin 1.75 mg/ml, and gramicidin 0.025 mg/ml (Neosporin), and an ophthalmic ointment of polymyxin B 5000 units/g, bacitracin 400 units/g, and neomycin 3.5 mg/g (Neosporin) were used topically as formulated.

The strains of *Pseudomonas* were susceptible to the antibiotics used by standard in-vitro tests (Davis *et al.*, 1977).

Results

For the trial presented in Table 1 animals were infected intracorneally, and treatment was begun

16 hours later. Topical therapy with antibiotics was more effective than therapy with saline ($P < 0.005$). Tobramycin 20 mg/ml, gentamicin 20 mg/ml, and a topical solution of polymyxin B, neomycin, and gramicidin were equally effective. Ointment containing the polymyxin B, bacitracin, and neomycin was significantly less effective than the topical solutions. Parenteral therapy with ticarcillin and carbenicillin was more effective than saline but less effective than topical therapy with antibiotic solutions ($P < 0.005$). Parenteral therapy with ticarcillin did not enhance the efficacy of topical therapy with tobramycin 20 mg/ml ($P > 0.1$).

In the trial shown in Table 2 therapy was begun

Table 3 Topical therapy trials of moderately severe experimental keratitis in guinea-pigs infected with 3 strains of *Pseudomonas aeruginosa**

<i>Pseudomonas</i> strain	Treatment	Corneal colony counts		P
		Mean $\pm$ SD	(N)	
107	0.15 M NaCl	6.21 $\pm$ 0.17	(6)	<0.005
	Tobramycin 20 mg/ml	1.38 $\pm$ 0.74	(6)	
	Polymyxin B, neomycin and gramicidin	5.00 $\pm$ 0.63	(6)	
110	0.15 M NaCl	4.55 $\pm$ 1.82	(4)	<0.005
	Tobramycin 20 mg/ml	1.06 $\pm$ 0.64	(5)	
	Polymyxin B, neomycin, and gramicidin	1.87 $\pm$ 2.56	(5)	
113	0.15 M NaCl	4.73 $\pm$ 1.00	(6)	<0.005
	Tobramycin 20 mg/ml	1.62 $\pm$ 0.94	(6)	
	Polymyxin B, neomycin and gramicidin	2.95 $\pm$ 0.87	(6)	

*Topical therapy of 2 drops every 30 minutes was begun 16 hours after infection and was given for 48 hours.

16 hours after infection and was continued for 24 hours. All antibiotic therapies were more effective than saline ($P < 0.005$). Topical therapy with solutions of tobramycin, gentamicin, amikacin, and a solution of polymyxin B, neomycin, and gramicidin were equally effective. The ointment containing polymyxin B, bacitracin, and neomycin was significantly less effective than the solutions ($P < 0.005$). Ticarcillin by intramuscular injection did not improve the efficacy of topical therapy with tobramycin in solution ($P > 0.1$).

Table 3 presents results of trials on 3 strains of *Pseudomonas* with tobramycin 20 mg/ml and a solution of polymyxin B, neomycin, and gramicidin. Tobramycin was significantly better than saline with all 3 strains. The solution of polymyxin B, neomycin, and gramicidin was not more effective than saline with strain 107. Tobramycin was more effective than the solution of polymyxin B, neomycin, and gramicidin with strain 107 ($P < 0.005$) but not with strain 110 ($P > 0.1$) or strain 113 ($P > 0.05$).

Discussion

In experimental keratitis in guinea-pigs antibiotic therapy was more efficacious when begun earlier than when begun later when the lesion was more severe (Davis *et al.*, 1978). Thus, therapy trials begun at 24 hours after infection constitute a more rigorous test of antibiotic therapy than trials begun at 16 hours after infection (Davis *et al.*, 1977). Trials begun at 16 hours after infection may possibly have greater clinical relevance than trials begun later, because an early abscess is beginning to form. Intuitively it seemed possible that some agents might

be more effective in moderately severe lesions and less effective for the later, more severe lesions. The results presented show that this is not so.

Considering the data shown here and our previous work, we conclude that the most consistently effective therapy of experimental *Pseudomonas* keratitis in the guinea-pig is an aminoglycoside antibiotic given topically in solution. The combination of polymyxin B, neomycin, and gramicidin was significantly less effective in several trials (Davis *et al.*, 1977). We concluded previously that polymyxin B alone in solution was significantly less effective than gentamicin or tobramycin in solution (Davis *et al.*, 1977). The ointment containing polymyxin B, bacitracin, and neomycin was inferior in efficacy to solutions of antibiotic. Intramuscular therapy with penicillins was less effective than topical therapy with aminoglycoside solutions in concentrations greater than 3 mg/ml. Intramuscular therapy with carbenicillin or ticarcillin did not significantly improve results of topical therapy with an aminoglycoside. In 1 trial, tobramycin, gentamicin, and amikacin were equally effective when applied topically in equal concentrations.

In brief, the most consistently effective therapy for experimental *Pseudomonas* keratitis in guinea-pigs is a topical aminoglycoside solution in the highest concentration that can be applied with safety (Davis *et al.*, 1977; Davis *et al.*, 1978). The relative efficacy of antimicrobial agents in this model did not change regardless of whether the therapy was started 16 or 24 hours after infection.

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