EFFECTS OF HABEKACIN, A NOVEL AMINOGLYCOSIDE ANTIBIOTIC, ON EXPERIMENTAL CORNEAL ULCERATION DUE TO *PSEUDOMONAS AERUGINOSA*

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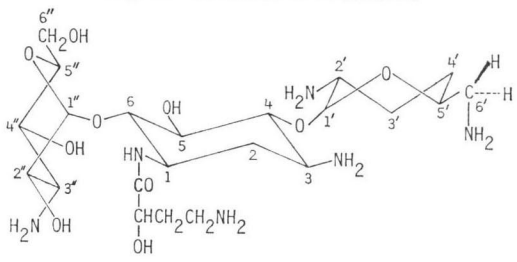
The effects of habekacin on corneal ulceration, caused by *Pseudomonas aeruginosa* IFO 3455, were studied in mice, in comparison with gentamicin and tobramycin. The minimal inhibitory concentrations of the antibiotics for the organism were: habekacin 2 μg/ml, gentamicin 2 μg/ml, and tobramycin 1 μg/ml. Habekacin showed protective and therapeutic effects on *Pseudomonas* keratitis. The 50% effective dose was approximately 1 μg per mouse, when the drug was topically applied three hours after the infection, and about 0.2 mg per mouse, when the antibiotic was intramuscularly injected one hour after the bacterial challenge to the cornea. Significant therapeutic and protective activities of habekacin were observed even by starting the topical and/or intramuscular treatment after the corneal ulcers were formed: *i.e.* 15 hours after the bacterial infection. Complete cure of *Pseudomonas* keratitis was found within a week in a number of the infected mice by both topical and systemic administrations of the drug. The protective and therapeutic effects of habekacin were comparable to those of gentamicin and tobramycin.

The corneal ulcer caused by *Pseudomonas aeruginosa* is a problem in terms of effective antibacterial therapeutics. Even with the administration of appropriate antibiotics, prompt destruction of the corneal stroma is observed, resulting in corneal perforation. Gentamicin and tobramycin have been reported to exhibit marked effects on experimental *Pseudomonas* keratitis[1,4,5], and are now clinically used for corneal infection caused by *Pseudomonas* organisms. However, increasing cases of corneal infections due to gentamicin- and/or tobramycin-resistant *Pseudomonas aeruginosa* have led to the investigation of new antibiotics for their effects on *Pseudomonas* keratitis.

Habekacin, 1-*N*-[(*S*)-4-amino-2-hydroxybutyryl]dibekacin, is a new aminoglycoside antibiotic, showing a broad antibacterial spectrum, including some of kanamycin-, gentamicin- and tobramycin-resistant organisms[2,3].

The effects of habekacin on corneal ulceration caused by *Pseudomonas aeruginosa* have been studied in mice, in comparison with gentamicin and tobramycin.

Fig. 1. Structure of habekacin.



Materials and Methods

Antibiotics

Habekacin was generously supplied by Dr. HAMAO UMEZAWA, Institute of Microbial Chemistry, Tokyo; and gentamicin and tobramycin by Shionogi & Co., Ltd., Osaka, Japan.

The Organism

A protease- and elastase-producing strain IFO 3455 of *Pseudomonas aeruginosa* was kindly supplied by Dr. J. Y. HOMMA, Kitasato Institute, Tokyo, Japan. The cells, grown overnight on heart infusion agar,were suspended in 50% skim milk, frozen and kept at −70°C. In each experiment, the frozen bacteria were brought to room temperature, and grown overnight on heart infusion agar. The successive culture, suspended in sterile saline (*ca.* 10^7 cells/ml), was used for the challenge to mice.

Sensitivity of *Pseudomonas aeruginosa* IFO 3455 to Antibiotics

The minimal inhibitory concentrations (MIC) were determined by liquid dilution method, using heart infusion broth. MICs were: habekacin 2 μg/ml, gentamicin 2 μg/ml, and tobramycin 1 μg/ml.

Animals

Seven week-old male *ddY* mice were employed, weighing about 25 g. Every eye was confirmed to possess no pathological changes by hand-held slit-lamp examination, prior to bacterial inoculation. In every experiment, each group consisted of six mice.

Inoculation of the Organism to Mice Cornea

Mice were anesthetized by intraperitoneal injection of 0.2 ml of 1: 10 diluted Nembutal (Abbott Lab.), containing 1 mg pentobarbital sodium. The cornea was incised in the central area at three spots with a subcutaneous needle (1/4 gauge) under a magnifying glass. The traumatized cornea was deposited once with a drop of about 0.01 ml of the above-described bacterial suspension, which contained approximately 10^5 cells. Sterile saline was applied in the same manner instead of the *Pseudomonas* inoculation, as control. The antibiotics were administered topically and/or intramuscularly, as described below; and control animals were treated with sterile saline instead of the drugs in the same procedure.

All the corneas were evaluated blindly for severity of damage, and graded as presented in Table 1.

Table 1. Grading scale for *Pseudomonas* keratitis.

Grade	Gross findings
0	No detectable damage.
I	Nubecula covering a part or the entire region of cornea.
II	Leukoma in the center of cornea, covering less than a half area of cornea.
III	Leukoma covering almost entire cornea with or without hypopyon.
IV	Complete damage of cornea usually associated with panophthalmitis.

Results

Mouse Protection by the Topical Application of Aminoglycosides against Corneal Damage Produced by *Pseudomonas aeruginosa* IFO 3455

Solutions of 9, 3 and 1 μg habekacin, 3 μg gentamicin, and 3 μg tobramycin, in 0.01 ml of saline, were topically applied three hours after the bacterial challenge. Six mice were used in each group. The pathological changes were observed 24 hours after the infection. As shown in Table 2, complete protection was found in 6 (100%), 5 (83%) and 3 (50%) mice in the groups administered with 9, 3 and 1 μg habekacin per mouse, respectively. Therefore, the ED_{50} value of habekacin seemed to be *ca.* 1 μg per mouse, when dropped onto the cornea. In simultaneous experiments, 3 μg of gentamicin or tobramycin showed complete prophylactic effects in all the mice. Thus, habekacin exhibited a significant protection against *Pseudomonas* keratitis to a similar degree as gentamicin and tobramycin.

Table 2. Mouse protection by topical application of antibiotics against corneal ulceration caused by *Pseudomonas aeruginosa* IFO 3455.

Antibiotic	μg/mouse	Pathological changes (Grade)				
		IV	III	II	I	0
None			2/6	4/6		
Habekacin	9					6/6
	3				1/6	5/6
	1				3/6	3/6
Gentamicin	3					6/6
Tobramycin	3					6/6

Table 3. Mouse protection by intramuscular administration of antibiotics against corneal lesion induced by *Pseudomonas aeruginosa* IFO 3455.

Antibiotic	mg/mouse	Pathological changes (Grade)				
		IV	III	II	I	0
None			4/6	2/6		
Habekacin	0.025		1/6	5/6		
	0.1			2/6	3/6	1/6
	0.4				2/6	4/6
	1.6					6/6
Gentamicin	0.2				2/6	4/6
Tobramycin	0.2				1/6	5/6

Protective Effects of Antibiotics on *Pseudomonas* Keratitis by Intramuscular Administration

Habekacin (1.6, 0.4, 0.1 and 0.025 mg), gentamicin (0.2 mg), and tobramycin (0.2 mg) in 0.05 ml were injected into the leg muscle (quadriceps), an hour after the *Pseudomonas* challenge to the corneas. Each group consisted of six mice. The corneal changes were observed 24 hours after the infection. Complete protection was found in 6 (100%), 4 (67%), 1 (17%), and 0 in the groups given 1.6, 0.4, 0.1 and 0.025 mg habekacin per mouse, respectively. All the animals injected with the antibiotic exhibited less grades of corneal damage than the control group. The ED_{50} value of habekacin (i.m.), therefore, was approximately 0.2 mg per mouse. In parallel experiments, the complete protective effects were observed in 4 mice (67%) with 0.2 mg gentamicin, and in 5 mice (83%) with 0.2 mg tobramycin (Table 3).

Therapeutic Effects of Aminoglycosides on *Pseudomonas* Ulceration by the Topical Application

The corneal damage of grade I or II was constantly observed 15 hours after bacterial challenge. Starting the treatment at 15 hours, the therapeutic effects of habekacin were studied. A drop (*ca.* 0.01 ml) of 40 or 3 mg/ml of the antibiotic solution was topically applied three times with an hour intervals every morning and evening. In simultaneous experiments, tobramycin was given in the same manner. The gross finding of pathological changes was recorded everyday (Table 4). The average of grading scales of corneal damage is illustrated in Fig. 2. Habekacin exhibited a significant therapeutic effect on a similar level as tobramycin. However, the complete cure of corneal lesion was not observed

Table 4. Therapeutic effects of aminoglycoside antibiotics on experimental *Pseudomonas* keratitis by the topical application.

Antibiotic	Grade of lesion	Day						
		1	2	3	4	5	6	7
None	IV					5/6	5/6	5/6
	III		5/6	6/6	6/6	1/6	1/6	1/6
	II	1/6	1/6					
	I	5/6						
	0							
Habekacin	IV							
0.4 mg×6/day	III		3/6					
	II	1/6	2/6	5/6	5/6	4/6	4/6	4/6
	I	5/6	1/6	1/6	1/6	2/6	2/6	2/6
	0							
Habekacin	IV							
0.03 mg×6/day	III		3/6	3/6	3/6	3/6	3/6	3/6
	II	1/6	2/6	2/6	2/6	2/6	2/6	2/6
	I	5/6	1/6	1/6	1/6	1/6	1/6	1/6
	0							
Tobramycin	IV							
0.4 mg×6/day	III		1/6					
	II	1/6	4/6	4/6	4/6	4/6	4/6	4/6
	I	5/6	1/6	2/6	2/6	2/6	2/6	2/6
	0							
Tobramycin	IV							
0.03 mg×6/day	III		3/6	2/6	4/6	3/6	3/6	3/6
	II	1/6	2/6	3/6	2/6	3/6	3/6	3/6
	I	5/6	1/6	1/6				
	0							

by the antibiotic treatment in a week.

Therapeutic Effects of Antibiotics on *Pseudomonas* Keratitis by Both Topical and Intramuscular Administrations

Since no complete cure could be obtained by the topical application of the antibiotics within a week, the drugs were given by both intramuscular and topical routes, starting 15 hours after the bacterial infection. The antibiotic solutions of 40 and 3 mg/ml were administered intramuscularly once a day with 0.05 ml, and topically with 0.01 ml twice a day. In parallel groups, tobramycin or gentamicin was given in the same procedure. The pathological changes of corneas were observed every day (Table 5). The average of grading scales of the corneal lesion is presented in Fig. 3. Habekacin showed significant therapeutic and protective effects to a similar degree as gentamicin and tobramycin. A complete cure was found in a mouse with a higher dose of habekacin or tobramycin, and in two mice with that of gentamicin.

Table 5. Therapeutic effects of antibiotics, by both topical and intramuscular routes, on corneal ulceration produced by *Pseudomonas aeruginosa* IFO 3455.

Antibiotic	Grade of lesion	Day						
		1	2	3	4	5	6	7
None	IV			2/6	2/6	2/6	2/6	2/6
	III		6/6	4/6	3/6	3/6	3/6	3/6
	II				1/6	1/6	1/6	1/6
	I	6/6						
	0							
Habekacin	IV							
daily	III							
2 mg i.m.	II							
0.4 mg×2	I	6/6	6/6	6/6	6/6	6/6	6/6	5/6
topically	0							1/6
Habekacin	IV							
daily	III					3/6	3/6	2/6
0.15 mg i.m.	II		4/6	5/6	5/6	2/6	2/6	3/6
0.03 mg×2	I	6/6	2/6	1/6	1/6	1/6	1/6	1/6
topically	0							
Tobramycin	IV							
daily	III							
2 mg i.m.	II							
0.4 mg×2	I	6/6	6/6	6/6	6/6	6/6	5/6	5/6
topically	0						1/6	1/6
Tobramycin	IV							
daily	III				1/6	1/6	1/6	1/6
0.15 mg i.m.	II			2/6	2/6	2/6	1/6	1/6
0.03 mg×2	I	6/6	6/6	4/6	3/6	3/6	4/6	4/6
topically	0							
Gentamicin	IV							
daily	III							
2 mg i.m.	II		1/6					
0.4 mg×2	I	6/6	5/6	6/6	6/6	5/6	5/6	4/6
topically	0					1/6	1/6	2/6
Gentamicin	IV							
daily	III				2/6	2/6	1/6	2/6
0.15 mg i.m.	II		2/6	2/6		2/6	2/6	1/6
0.03 mg×2	I	6/6	4/6	4/6	4/6	2/6	3/6	3/6
topically	0							

Discussion

The current results suggest that habekacin can be used in corneal infections of *Pseudomonas aeruginosa* as can gentamicin and tobramycin. Habekacin is effective against some gentamicin- and/or tobramycin-resistant bacteria[2,3]. Early antibiotic treatment is required to minimize the corneal

Fig. 2. Comparison of therapeutic effects of topical application of aminoglycosides on corneal ulceration, caused by *Pseudomonas aeruginosa* IFO 3455.

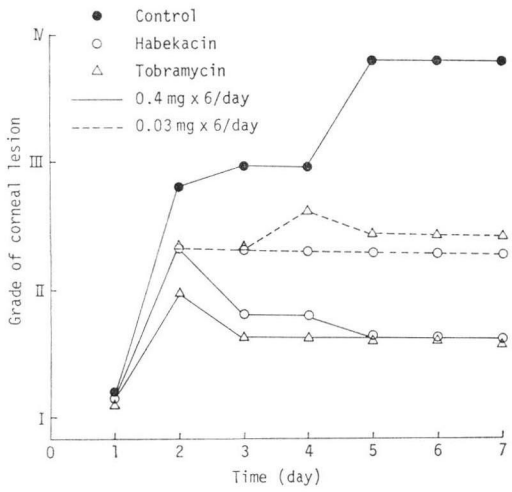
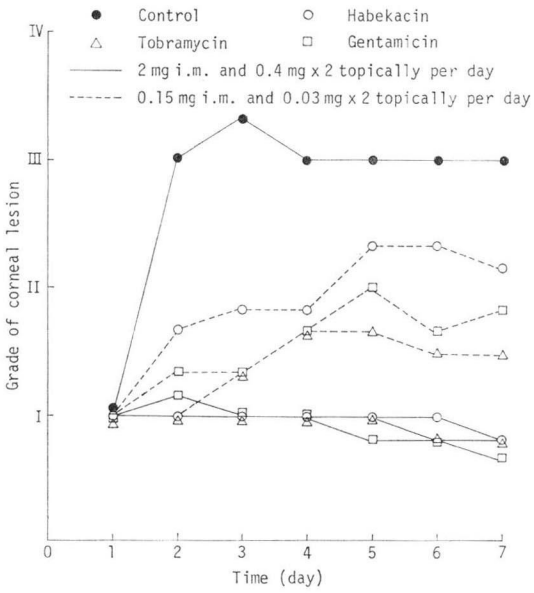


Fig. 3. Comparison of therapeutic effects of topical and intramuscular administrations of aminoglycosides on *Pseudomonas* keratitis.



damage. However, no corneal perforation and/or panophthalmitis were observed in those cases in which the administration of habekacin was started after the formation of corneal ulcers. Histologic studies showed that pathological changes were limited to the cornea in habekacin-treated cases. In contrast, the changes were extended to intraocular infections in the control eyes. Corneas treated with the antibiotic were culture-negative for *Pseudomonas aeruginosa* on day 7, and bacteria were not detected in Gram stains of the tissue sections (Data are not shown).

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