JOURNAL OF OCULAR PHARMACOLOGY
Volume 9, Number 3, 1993
Mary Ann Liebert, Inc., Publishers

# Continuous Flow Perfusion of Gentamicin with a Scleral Shell Reduces Bacterial Colony Counts in Experimental *Pseudomonas* Keratitis

D.S. ROOTMAN[1] and M. KRAJDEN[2]

*Departments of [1]Ophthalmology and [2]Microbiology, University of Toronto, Toronto, Ontario, Canada*



## ABSTRACT

We have previously shown the pharmacokinetic value of delivering gentamicin to the rabbit anterior segment using the Morgan Therapeutic Lens™. The present study utilized an intrastromal injection model of Pseudomonas keratitis to test the therapeutic efficacy of continuous flow delivery of gentamicin with the Morgan therapeutic lens. All eyes (n = 52) received an intrastromal injection of approximately 1800 colony forming units (CFU) of Pseudomonas aeruginosa. At 22 hours after injection, eyes were perfused for 6 hours with saline or gentamicin (1, 2.5 or 5mg/ml), or received gentamicin drops (13.6mg/ml) at 15 minutes for four doses, then hourly for 6 hours. Corneas were homogenized and plated to determine bacterial survival, and expressed as log colonies (CFU). Log CFU recovered were 7.37 ± 0.04, 6.64 ± 0.20, 5.64 ± 0.31, and 3.56 ± 0.50 log CFU for saline perfusion, 1, 2.5, 5 mg/ml gentamicin perfusion respectively. Following six hours of treatment with topical fortified gentamicin drops, 5.93 ± 0.34 log CFU were recovered. Gentamicin perfusion (5 mg/ml) was significantly different from saline or the other treatment groups ($P<0.05$). Continuous corneal perfusion with the Morgan Therapeutic Lens™ demonstrated an increasing dose response curve with increasing perfusate concentration. It was effective in the treatment of experimental Pseudomonas keratitis.

## INTRODUCTION

Bacterial keratitis can often present a difficult problem for the treating physician. Delay in treatment can lead to scarring of the cornea and sometimes to loss of the eye. The incidence of this disease has been steadily on the increase due to the common usage of contact lenses. Studies have shown that increasing lens wearing time proportionally increases the risk of developing keratitis.[1] Pseudomonas aeruginosa is a ubiquitous gram negative bacteria that has emerged as one of the common organisms involved in the etiology of contact lens related microbial keratitis.[2-5]

Currently, bacterial keratitis is treated by the instillation of commercial and specially prepared fortified antibiotics drops.[6-8] Under many circumstances, the patient is admitted to hospital to facilitate the frequent administration of topical drops every half hour. This is labour intensive for the nursing staff and requires a hospital admission. Other methods of treating bacterial keratitis have included iontophoresis, whereby drug delivery to the cornea is facilitated by the application of a weak electrical current across a solution of antibiotic.[9-11] Collagen shields have also been shown to be pharmacokinetically and therapeutically useful in delivering drugs to the cornea.[12-14]

The Morgan Therapeutic Lens (MTL) is a smooth scleral shell made out of polymethylmethacryalate. It is attached to a silicone tube which can be connected via IV tubing to a fluid source. It was originally developed by Dr. Morgan for ocular lavage in the treatment of chemical injuries to the eye.[15] We have previously shown the pharmacokinetic efficacy of the Morgan Therapeutic Lens in delivering gentamicin to the cornea and aqueous humor.[16] Additionally, this lens has been shown to be effective in delivering epithelial growth factor to the cornea.[17] The present study was designed in order to compare continuous flow of gentamicin via the Morgan Therapeutic Lens in the treatment of experimental Pseudomonas keratitis in the rabbit to standard treatment of frequent application of fortified topical drops.



## MATERIALS AND METHODS

Treatment of the rabbits in this investigation were in compliance with the ARVO resolution on the use of animals in research.

New Zealand white rabbits (2-3kg) were utilized for all experiments. All treatments were performed with the rabbits anesthetized by a 2ml intramuscular injection of a 5:4 mixture of 100ml/ml of ketamine (Rogarsetic™, Rogar/stb, London, Ontario) and 20mg/ml of xylazine (Rompun™, Haver, Etobicoke, Ontario). A single drop of 0.5% proparacaine (Ophthetic™, Allergan Inc., Pointe Claire, Quebec) was applied to the cornea. One eye of each rabbit received an intrastromal injection of 10 ul containing 1830 ± 240 CFU Pseudomonas aeruginosa (ATCC strain 27853). The bacteria were grown to an early log phase in tryptic soy broth at 37°C for four hours and then adjusted to the appropriate concentration with sterile saline using a 0.5 MacFarland standard. The number of bacteria injected was retrospectively verified by serial tenfold dilution plating on blood agar. Inoculation was done by inserting a 30 gauge needle intrastromally close to the centre of the cornea. The infection was allowed to progress for twenty hours. Eyes were randomly assigned to one of the following treatments: Group 1 eyes received saline perfusion. Group 2 eyes received fortified gentamicin topical drops (13.6mg/ml, 0.68mg/50 ul) applied every 15 minutes in the first hour and once hourly thereafter for a total of six hours. Group 3 eyes received gentamicin perfusion at a concentration of 1 mg/ml. Group 4 eyes received gentamicin perfusion at a concentration of 2.5 mg/ml. Group 5 eyes received gentamicin perfusion at a concentration of 5 mg/ml. All perfusions were performed for a total of 6 hours at 2ml/hour. There were 10 or 11 eyes in each group. Gentamicin drops were prepared by adding 2ml of 40mg/ml gentamicin sulfate (Cidomycin™, Roussel Canada Inc., Montreal, Quebec) to 5ml of 0.3% gentamicin drops (Garamycin). All perfusion experiments were done under maintenance general anesthesia using the intramuscular anesthetic described above.

At the end of six hours, the rabbits were sacrificed by an intravenous overdose of T-61 (Hoechst Canada Inc., Montreal, PQ.) The

cornea from each animal was rinsed with 3ml sterile phosphate-buffered saline and removed by incision at the corneoscleral limbus. Corneas were minced into small pieces and homogenized in 3ml PBS by a tissue homogenizer under sterile conditions. The tissue was ground to a fine suspension. An aliquot, 0.5ml, of the homogenate was serially diluted with sterile saline and 0.1ml from the respective dilutions or undiluted homogenate were plated on blood agar plates in triplicate. The plates were incubated at 37°C for 20-22 hours, and the number of CFU per plate was determined and expressed as Log CFU/cornea. T-tests between the means from each treatment group were conducted for statistical comparison.

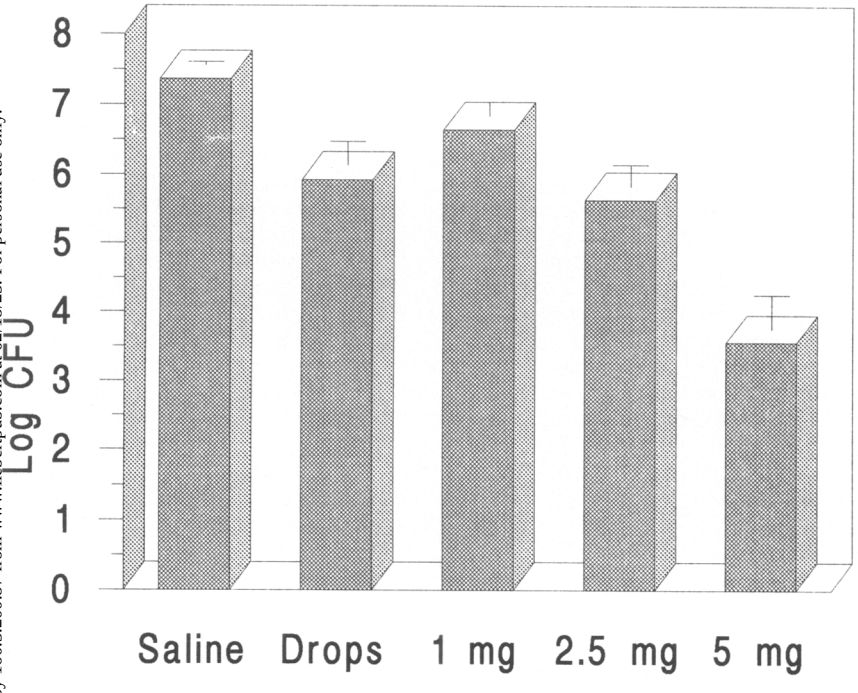


Figure 1. Comparison of CFU's recovered following treatment. Gentamicin drops, 13.6 mg/ml, every 15 min X 4, then every hour. Saline; Gentamicin, 1 mg, 2.5 mg, 5 mg were perfused for 6 hours.



## RESULTS

See Figure 1 where the results of the experiments are presented. In the eyes receiving saline perfusion, 7.37 ± 0.4 log CFU were recovered. With gentamicin perfusion, at concentrations of 1, 2.5 and 5 mg/ml, 6.64 ± 0.20, 5.64 ± 0.31, and 3.56 ± 0.50 log CFU were recovered. Following six hours of treatment with topical fortified gentamicin drops, 5.93 ± 0.34 log CFU were recovered. There was a significant difference between the untreated eyes and those receiving gentamicin at all concentrations studied via perfusion or via topical drops. The Gentamicin perfusion, (5 mg/ml) was significantly different than all the other treatment groups. Topical drops were significantly different than 1mg/ml gentamicin perfusion. Perfusion with 2.5 mg/ml was different than 1 mg/ml perfusion.

## DISCUSSION

Dr. Hessburg, many years ago, described a continuous perfusion technique whereby a tube was implanted through the lid and connected to a continuous drip system. This system provided lavage and effective treatment for Pseudomonas keratitis.[18-20] This technique, however, has not become widely used due to the necessity for surgical implantation of the tube. Other investigators have used novel methods of attaching the tube to the skin surrounding the eye.[21] The Morgan Therapeutic Lens has been used for ocular lavage for many years. However, its potential as an ocular drug delivery system has not been fully investigated. It has the advantages of being comfortable due to its smooth configuration, and well tolerated by the patients. It can be attached to an electronic intravenous pump and therefore facilitates nursing care. A small portable pump can also be attached to the lens allowing the patient to be more mobile during treatment.

Our previous studies have shown the efficacy of using the Morgan lens to deliver gentamicin to the cornea and aqueous humor. This is the first study, to our knowledge, which investigates the therapeutic efficacy of continuous flow gentamicin via the Morgan lens in the treatment of experimental Pseudomonas keratitis. Our results show that after a treatment period of six hours, the number of bacteria was significantly reduced via the Morgan Therapeutic Lens as compared to treatment with drops or the untreated control. The system offers the advantage of providing continuous lavage, ie. removing the infected debris from the corneal surface. The solution provides a continuous diffusion gradient from outside towards in and may thus be more effective in delivering the drug so that lower concentrations of the drug may be used to reduce the endothelial toxicity caused by high concentrations of antibiotics delivered either topically or subconjunctivally.

In conclusion, the Morgan Therapeutic Lens is an effective means of treating experimental Pseudomonas keratitis. Further studies are underway to assess the toxicity of delivering antibiotics via this route.



## ACKNOWLEDGEMENTS

Supported by MRC Grant MA 10947 (Dr. Rootman) and a grant from Mortan Corporation, Missoula, Montana

## REFERENCES

1. Schein, O.D., Glynn, R.J., Poggio, E.C., Seddon, J.M., Kenyon, K.R. The relative risk of ulcerative keratitis among users of daily-wear and extended wear soft contact lenses. New England Journal of Medicine., 321:773-8, 1989.

2. Weissman, B.A., Mondino, B.J., Petit, T.H., Hofbauer, J.D. Corneal ulcers associated with extended-wear soft contact lenses. Am J Ophthalmol., 97:476-481, 1984.

3. Hassman, G., Sugar, J. Pseudomonas corneal ulcers with extended-wear soft contact lenses for myopia. Arch Ophthalmol., 101:1549-1550, 1983.

4. Adams, C.P., Cohen, E.J., Laibson, P.R., Galentine, P., Arentien, J.J. Corneal ulcers in patients with cosmetic extended-wear contact lenses. Am J Ophthalmol., 96:705-709, 1983.

5. Shivitz, I.A. Bilateral simultaneous Pseudomonas keratitis with myopic extended-wear contact lenses. Ann Ophthalmol., 19:204-206, 1987.

6. Kupferman, A., Leibowitz, H.M. Topical antibiotic therapy of Pseudomonas aeruginosa keratitis. Arch Ophthalmol., 97:1699-1702, 1979.

7. Davis, S.D., Aarff, L.D., Hyndiuk, R.A. Comparison of therapeutic routes in experimental Pseudomonas keratitis. Am J Ophthalmol., 87:710-716, 1979.

8. Leibowitz, H.M., Ryan, W.J., Kupferman, A. Route of antibiotic administration in bacterial keratitis. Arch Ophthalmol., 99:1420-1423, 1981.

9. Rootman, D.S., Hobden, J.A., Jantzen, J.A., Gonzalez, J.R., O'Callaghan, R.J., Hill, J.M. Iontophoresis of tobramycin for the treatment of experimental Pseudomonas keratitis in the rabbit. Arch Ophthalmol., 106:262-265, 1988.

10. Rootman, D.S., Jantzen, J.A., Gonzalez, J.R., Fischer, M.J., Beuerman, R., and Hill, J.M. Pharmacokinetics and safety of transcorneal iontophoresis of tobramycin in the rabbit. Invest Ophthalmol Vis Sci., 29:1397-1401, 1988.

11. Hobden, J.A., Rootman, D.S., and O'Callaghan, R.J. Iontophoretic application of tobramycin to uninfected and Pseudomonas aeruginosa-infected rabbit corneas. Antimicrob Agents Chemother., 32:978-981, 1988.

12. Unterman, S.R., Rootman, D.S., Hill, J.M., Parelman, J.J., Thompson, H.W., and Kaufman, H.E. Collagen shield drug delivery: Therapeutic concentrations of tobramycin in the rabbit cornea and aqueous humor. J Cataract Refrac Surg., 14:500-504, 1988.

13. Phinney, R.B., Schwartz, S.D., Lee, D.A., and Mondino, B.J. Collagen shield delivery of gentamicin and vancomycin. Arch Ophthalmol., 106:1599-1604, 1988.

14. O'Brien, T.P., Sawusch, M.R., and Dick, J.D. Use of corneal collagen shields versus soft contact lenses to enhance penetration of topical tobramycin., J Cataract Refract Surg. 14:505-507, 1988.

15. Fernandez, M.B. Eye irrigating lenses. Arch Emerg Med., 8:274-276, 1991.

16. Rootman, D.S., Willoughby, R.P.N., Bindlish, R., Avaria, M., Basu, P.K., and Krajden, M. Continuous flow contact lens delivery of gentamicin to rabbit cornea and aqueous humor. J Ocular Pharmacol., 8:317-323, 1992.

17. Wedge, C., Sheardown, H., Chou, L., Cheng, Y.L., Rootman, D.S. The effects of continuous perfusion delivery of epidermal growth factor on corneal epithelial wound healing. Invest Ophthal Vis. Sci., (Suppl.) 33:889, 1992.

18. Hessburg, P.C. Treatment of Pseudomonas keratitis in humans. Amer J Ophthal., 61:896-903, 1966.

19. Hessburg PC, Truant JP, Penn WP. Treatment of Pseudomonas keratitis in rabbits. Amer J Ophthal., 61:49-54, 1966.

20. Hessburg PC. Management of Pseudomonas keratitis: Therapeutic review. Survey of Ophthal., 14(1):43-54, 1969.

21. Snyder, R.W., Leering, P., Hyndiuk, R.A., Konig, S.B., McDermott, M.L. Continuous antibiotic delivery (CADS). Invest Ophthalmol Vis. Sci. (Suppl)., 92:439, 1988.

Received: April 1, 1993
Accepted for Publication: May 21, 1993

Reprint Requests: D.S. Rootman, M.D.
Department of Ophthalmology
1 Spadina Crescent
Toronto, Ontario M5S 2J5, Canada