J. Kor. Pharm. Sci., Vol. 22, S35-S47 (1992)

Hydrogel Ocular Inserts for the Treatment of Infectious Bovine Keratoconjunctivitis

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(Received April 4, 1992)

Abstract

Hydrogel coated ring shaped ocular inserts (containing the antibiotic, tylosin tartrate) were used in an evaluation of the effectiveness of polymeric ocular drug release devices for treating infectious bovine keratoconjunctivitis. The *in vivo* experiments represent the first experiments using hydrogel ocular inserts containing an antibiotic for treating infectious bovine keratoconjunctivitis.

In the infection tests, ten calves were challenged with $2.4\times10^8\sim1.6\times10^9$ *Moraxella bovis* (a bacterium) colonies per eye following two ten minute ultraviolet radiation eye preconditioning exposures. Ninety five percent of the eyes (19 of 20 eyes) were successfully infected by this method. All infected eyes were monitored for the presence of the bacteria quantitiatively, and clinical observations were made for 14 days. The test was performed by three consecutive steps: 1) inoculation with 2 ultraviolet (UV) radiations, 2) growth of bacterial colonies and 3) treatment with medicated ring-shaped devices. The first bacteriological measurements after 2 UV exposures were performed at day 3 of the tests. At day 7 after inoculation of both eyes of a calf with *M. bovis*, a medicated or a non-medicated ring-shaped device was inserted into each eye of a calf. The eye receiving the non-medicated ring was taken as a control for comparison with the eye that received a medicated ring. During the next 7 day period following a medicated ring insertion, the number of bacteria in the treated eyes dropped dramatically to negligible levels (0 to 30 colony forming units/swab), while the control eyes which received a non-medicated ring still exhibited a relatively high number of bacteria (10^3 to 10^6 colony forming units/swab). The number of bacteria was significantly reduced by the antibiotic released from the medicated ocular insert.

1. INTRODUCTION

Infectious bovine keratoconjunctivitis (IBK), commonly called "pinkeye", is an extremely contagious eye disease in cattle. Once this disease has entered the herd, all animals should be treated. Many organisms have been associated with IBK, but only the bacterium, *Moraxella bovis*, has been shown to cause of this disease by itself (Barner, 1952), *M. bovis* is a short, gram-negative, non-motile rod with rounded ends in pairs or in short chains (Hughes, 1981). IBK is a disease occurring perennially wherever cattle are raised. However, pinkeye is highly prevalent, particularly in summer months and has a high incidence in beef cows and calves, stock cattle and replacement females. Transmission of the infection can occur by direct contact with ocular secretions, nasal secretions, or by face flies. Cattle with this disease exhibit heavy tearing, swollen eyelids and eyes that are very sensitive to sunlight in the initial stages. Then, conjunctivitis, keratitis, corneal ulceration and scarring appear in the late stage (Gelatt, 1981). IBK is a costly eye di-

sease. The disease is rarely fatal, but it may result in a major economic loss associated with weight loss in beef herds, a reduction in milk production in dairy herds, delays of breeding, the cost of treatment and a reduced sale price. It has been estimated by U.S. Department of Agriculture (1976) that the U. S. beef industry lost more than 150 million dollars each year.

The present treatment method consists of topical applications of antibiotics and sulfonamide of eye drops, ointments, or powders for a period of five to seven days. However, since tears rapidly wash the drug from an eye, therapeutic levels of drug cannot be maintained during the therapy, and drugs must be applied repeatedly. Subconjunctival and intramuscular injections (Pugh and McDonald, 1977) of antibiotics are also used as the treatment methods to suppress the disease, but repeated medications are necessary. Hughes *et al.* (1979) compared the efficacy of vacination with antibiotic treatment. They concluded antibiotic treatment was more effective as a prophylaxis against IBK infection than vaccination. Since an effective vaccine is difficult to produce, the use of good husbandry techniques and treatment with antibiotics may offer a better alternation to control IBK than by using vaccinations (Hughes *et al.*, 1979; Pugh *et al.*, 1982). Third eyelid flaps (Anderson *et al.*, 1976) and tarsorrhaphy (suturing the upper and lower eyelid together) are frequently used for the treatment of severe ulceration keratitis in order to preserve the eye. As an alternative method to treat ocular infections, the controlled release systems of antibiotics have been studied in order to release drug continuously. The eye has been considered to be a useful site for using controlled release systems. Compared to other body sites, it is relatively easy for divices to be inserted into the eye and to be removed from the eye. Three types of ocular devices were studied as a treatment system to treat IBK: terramycin eye pellet (Hawley, 1954), biodegradable ocular inserts (Theodorakis *et al.*, 1983), and soluble and insoluble collagen films (Slatter *et al.*, 1982a; Punch *et al.*, 1985) of antibiotics. However, none of these devices had enough *in vivo* data and maintained therapeutic levels (above 1.2 μg/hr) of drug release for the desired period (over 7 days). Also, these ocular inserts exhibited poor retention characteristics due to improper rigidity or flexibility properties.

Hughes and Pugh (1975; 1980) used ring-shaped plastic tubes in the bovine eye in preliminary retention tests. However, the tubular inserts did not contain any drugs. They determined introductory information on the size and shape of polymer rings for potential use in the bovine eye. Related research using ring-shaped ocular inserts has been conducted (Greer and Ryoo, 1987; Punch *et al.*, 1987 b). In 1986, a ring-shaped device releasing tylosin tartrate (contained in hydrogel coatings on polyvinyl chloride tubes) has been studied in a series of *in vitro* experiments (Ryoo, 1986). All of three types of polymer tubes (silicone rubber, Teflon® and polyvinyl chloride) were fabricated using a hydrogel coating. The hydrogel did not cause any eye irritation and was shown to be capable of sustaining the drug release of the antibiotic for the required period of interest for IBK treatment applications. The physical characteristics of three tubings were compared and evaluated to make a suitable ring device for use in *in vivo* tests. Among three ring devices, an ocular insert using polyvinyl chloride tubing was chosen as the best device material because it had good flexibility, was easy to fabricated in the form of a ring device, was low in cost, and had good mechanical properties. Also in these *in vitro* experiments, drug release rates monitored (*in vitro* experiments using an artificial tear solution) were observed to be above the therapeutic level necessary for suppressing the bacterium (*Moraxella bovis*) for at least 9 days.

On the basis of these *in vitro* release results, the present work was evaluated for the effectiveness of hydrogel ocular inserts in *in vivo* experiments of two types: (1) *in vivo* drug release tests of ocular inserts and (2) clinical studies using medicated ring devices for the treating infected eyes and non-medicated ring devices for use as controls.

2. MATERIALS AND METHODS

2-1. Fabrication of Hydrogel Ocular Inserts

The methyl methacrylate (Aldrich Chemical Co., Milwaukee, WI) and hydroxyethyl methacrylate (Polyscience Inc., Washington, PA) copolymers (90 : 10 molar basis) were made in a batch process (Greer and Ryoo, 1987). Two types of monolithic ring devices were made: medicated and non-medicated rings. A medicated ring was made by dip-coating a polyvinyl chloride tube (Becton, Dickinson and Co., Rutherford, NJ). The ring-shaped tube was dipped into a mixture of 1 g of copolymer, 0.5 g of tylosin tartrate (Sigma Chemical Co., St Louis, MO) and 20 m*l* of dimethyl formamide to provide a coating of hydrogel containing a total of 50 mg of tylosin tartrate. A non-medicated ring was made using dip coatings of a solution containing 1.5 g of copolymer and 20 m*l* of dimethyl formamide. After complete drying, both sides of a polymer ring device were then connected using a short piece of a thin radiopaque polyvinyl chloride tubing (i.d., 0.051 cm×o.d., 0.091 cm). A small bead of drug/copolymer mixture was used as an adhesive to connect the tube sides. A non-medicated ring device contained only about 150 mg of hydrogel. The diameters of complete rings were within the range of 30 to 60 mm. This provides a number of rings for use with different size animals. The ring size used in the *in vivo* test was about 50 millimeters.

2-2. *In vivo* Drug Release Test of Ocular Inserts

A cotton-tipped swab was used to absorb tear fluid at the medical canthus area of the eye (healthy cows). A swab with a wood stick was retained for a while at the medial canthus to obtain sufficient tears (about 0.2~0.3 m*l*) so as to be able to analyze for drug release amounts. Then, the tear fluid in a swab was withdrawn from the swab by suctioning with an 1 m*l* tuberculin syringe having a 27 gauge needle. Portions of this sample were spotted directly on the thin layer chromatography (TLC) plate in the field or at the laboratory. Some samples which contained small amounts of drug required multiple spotting to yield enough drug to make a visible spot. The drug concentration of tear samples was analyzed by using a TLC technique. The TLC plate (Whatman Chemical Separation Inc., Clifton, NJ) spotted by standard and tear samples (2.5 μ*l* per each sample) was developed in 85% methanol-15% water and then was air-dried completely. Then, a TLC plate was sprayed with a 10% H_2SO_4-90% methanol solution, and was heated for 5 min at 100℃. The plate, where the spots could be visualized, was analyzed using a Kontes Fiber Optics Scanner (model 800) and a computer analysis system. The computer analysis system was developed to characterize the amount of drug release from the TLC plate on which tear samples were spotted. This system consisted of two parts: hardware and software. Hardware included a personal computer (IBM PC-AT or Zenith 248) and data acquisition system (Keithley system 570). Software was composed of two programs: data acquisition system and data processing system for quantitating the drug amount released from ocular inserts to eyes. Using this computer analysis system (Ryoo *et al.*, 1989), the peak data were analyzed automatically within about 3 min for 20 samples/plate and saved on a floppy disk for post-run analysis.

2-3. Animals

Ten Holstein calves were used. They had not been previously exposed to IBK and

were raised on antibiotic-free calf feed. The ages of the calves were in the range of 3 to 6 months. They were kept indoors in individual isolation units and were excluded from sunlight and flies. The quarters were mechanically ventilated with filtered air. Studies were performed in groups of 2 or 3 calves.

2-4. Antibiotic Susceptibility Tests

A lawn of *M. bovis* was prepared by the same procedures as described in the following sections on preparation of *M. bovis* seed (bacterial cultures). Then, the antibiotic disks were placed on the *M. bovis* lawn on a blood agar plate (BAP). Each disk containing a different antibiotic was tested. The plates were stored in the incubator for 24 hrs at 37℃ in 10% CO_2. Then, they were stored at room temperature for 24 hrs. The diameter of zones of growth inhibition associated with the effect of each antibiotic was measured to evaluate the sensitivity of the antibiotic.

2-5. Bacterial Cultures

An isolate of *M. bovis* (118F) was obtained from a herd with severe IBK (Rosenbusch, 1983; Ostle and Rosenbusch, 1986). Strain 118F of *Moraxella bovis* grown as a 5th passage was used as a seed. One cryotube containing the *M. bovis* strain stored in a −70℃ freezer, was defrosted in warm water. A starter BAP was incubated by streaking *M. bovis* using a swab, and put into the sealed Bio-Bag® system to provide a microaerophilic atmosphere (10% CO_2). Then, the plates was incubated for 24 hrs at 37℃. A half of the *M. bovis* colonies (smooth corroding hemolytic type) on the plate left at room temperature for 24 hrs were harvested by using a sterile cotton swab. Then, the bacteria on the cotton swab were resuspended in 1.0 m*l*～1.5 m*l* of 10% $MgCl_2$ solution. One tenth milliliter of this suspension was spread on each blood agar plate with a sterile bent Pasteur pipette. The number of the plates was determined by the number of experimental animals (usually, twice as many as the number of animals). These plates were incubated for 22 hrs in 10% CO_2 in air at 37℃.

2-6. Inoculation Procedures

Two sunlamps (General Electric Co., Model RSK6, Nela Park, Cleveland, OH; The wavelength range is 2800～3000Å) were used as sources of ultraviolet (UV) radiation. Irradiation was accomplished by 2 UV exposures (for 10 min at 61 cm) given 1 day before and just before inoculation with *M. bovis* inoculum. After second UV irradiation, a *M. bovis* inoculum was prepared from the incubated blood agar plates. All bacteria from each plate were harvested and suspended in 2 m*l* of cold gram-negative wash solution [0.5 M sucrose, 5 mM phosphate buffer (pH 7), 50 mM $MgCl_2$, and 0.05% bovine serum albumin]. One tenth milliliter of the suspension was saved in a tube in ice for the titration of *M. bovis* inoculum. An aliquot (1 m*l* for an eye) of *M. bovis* challenge was poured in a separate tube and was then kept on ice for 30 min. Each inoculum was instilled in each eye with a sterile cotton swab. About 0.5 m*l* of the inoculum was absorbed when a swab was soaked for a while. The reminder of the inoculum (approximetely 0.5 m*l*) was then poured into the eye. The bacterial concentration contained in each inoculum was estimated approximately 2.4×10^8～1.6×10^9 colony-forming units/m*l* (CFU/m*l*).

2-7. Bacteriological Evaluation

After a *M. bovis* challenge exposure, each tear sampling was repeated at 2 day-intervals for 17 days in group I and for 15 days in groups II, III and IV. A sterile cotton swab was used to collect a tear sample from each animal. Each swab was immersed in a centrifuge tube containing 3 m*l* of ice-cold gram-negative wash solution and was agitated. The swab was pulled out with a sterile forceps after squeezing most of the fluid out of the swab into the tube. Later, all of the tubes were spun in the centrifuge at 12,000 revolutions per minute for 10 min at 4℃. The supernatant was poured out gently and 0.3 m*l* of

gram-negative wash or 10% $MgCl_2$ solution was added to the tube containing the solid pellet. From this suspension (0.3 m*l* of undiluted sample), 0.1 m*l* of undiluted sample was added to a BAP. Then, another 0.1 m*l* of undiluted sample was added to 2.9 m*l* of diluent (gram-negative wash or 10% $MgCl_2$ solution) to make 1/30 of dilution. Also, 1/3000 of dilution was made by mixing 0.1 m*l* of 1/30 dilution with 9.9 m*l* of diluent. One tenth milliliter from diluted samples (1/30 and 1/3000) was also added to individually separated BAP. These samples represent 1/3, 1/90 and 1/9000 of the swab burden per eye, respectively. These samples were made in order to count the total number of *M. bovis* colonies accurately. The plates were incubated for 24 hrs at 37℃ with 10% CO_2 in air, and the number of *M. bovis* colonies were read at 24 hrs. The results were expressed as the logarithm of the number of colony-forming units/swab or $\log_{10}$ CFU/swab.

2-8. Clinical Examination

All calves were observed once a day to check for clinical signs of pinkeye by visual examination. Any clinical signs of eye irritation of obvious change in tear flow rates were reported. The clinical condition was assessed on the basis of the progress of the disease. Five stages of the disease were categorized: initial stage-photophobia, epiphora, blepharospasm; conjunctivitis; keratitis; central corneal ulceration; perforation of corneal ulcer, corneal scarring blindness.

2-9. Treatment of Ocular Inserts for Infected Calves

Seven days after *M. bovis* was instilled into the eye, one of two types of ocular inserts (medicated and non-medicated) was inserted into an infected eye. Both eyes were used, one as a treated eye (using a medicted ring) and one as a control eye (using a non-medicted ring). All of the rings were selected on the basis of the eye size of each animal by using the criterion of being 4 to 10 mm larger than the side-to-side dimension of the palpebral aperture of the eye. The rings were preconditioned in Ringer's solution for an hour. After inserting the ocular inserts, the eyelids of the calves in groups I, II, and III were sutured (single suture for third and upper eyelid, and double suture for upper and lower eyelid). Suturing was done for both eyes to insure that no rings would be lost during this experiment. However, eyelids in group IV were not sutured in order to check whether or not rings were retained for at least a week. Animals were sedated with xylazine (Rompun®, haver-Lockhart, Shawnee) prior to suturing. After suturing, the suture condition of each eye was observed daily in order to see if additional suture is needed.

2-10. Post-Treatment for Control Eyes

About a week later, all sutures and ocular inserts remained in the eye were removed. Rings were gently washed with distilled water and dried in a desiccator. After drying, rings were weighed to measure the amount of drug released during the experiment period. To treat the infections of the control eyes, medicated ring devices were inserted in place of the control rings with an immediate injection of 1800 mg of long-acting oxytetracycline (Liguamycin®, Pfizer Inc., Newport, NY) (only injection for group I and both of medicated ring insertion and injection for groups II and III). However, for group IV, 0.7 m*l* of Azimycin® (Schering, Union, NJ) was given as a subconjunctival injection (commonly used in field cases of pinkeye) to provide a comparison of a field treatment method with that of the treatment method using medicated ocular inserts.

3. RESULTS

3-1. *In vivo* Drug Release Test of Ocular Inserts

The release rate of tylosin tartrate measured using tear samples is shown in Table I. The required minimum drug release rate was estimated as 1.2 to 2.4 μg/hr in the pre-

Table I—Drug Release Rate with Time for *in vivo* Testing

Ring No.	Time (days)							
	1	2	3	4	5	6	7	8
VI-42-15	160.6[a]	25.8	37.9	nt*				
VI-44-02	14.9[b]	nt						
VI-45-01	19.4[b]	54.6	41.1	nt				
VI-43-02	10.5	15.1	14.7	28.0	nt			
VI-44-01	7.9[b]	7.1	nt					
VI-46-03[c]	—	—	23.0	11.8	22.2	20.6	30.0	nt

[a]Release rate in μg/hr. [b]These samples might be mixed with the large amount of tears. [c]This ring was inserted in the same eye after VI-44-01 fell out. *nt : Ring devices fell out, so further data could not be tested.

Table II—Susceptibility and Zone of Growth Inhibition

Name of antibiotic	Reaction	Diameter of zone of growth inhibition
Tylosin tartrate	sensitive	4 mm
Ampicillin	sensitive	5 mm
Streptomycin	resistant	0 mm

vious work (Ryoo, 1986). All of the *in vivo* release rate data in Table I were seen to be above the minimum drug release rate necessary to suppress IBK in these cattle. The release rates were found to be in the range of 7～160 μg/hr. According to the above re-

Table III—Retention Characteristics of Ocular Inserts

Group No.	Animal No.	Size (mm)		etention time (days)									
		eye[a]	ring[b]	0	1	2	3	4	5	6	7	8	9
I	149L	39.5	46.64	IS[c]		P					R		
	R	39.5	46.25	IS							R		
	150L	36.5	45.78	IS		P					R		
	R	36.5	45.25	IS		O					R		
II	152L	35.0	44.8	IS							R		
	R	35.0	45.0	IS		F/IS			O		R		
	153L	36.0	45.5	IS		O/S		O			R		
	R	36.0	45.4	IS		O/S					R		
	154L	36.0	45.5	IS		O/S					R		
	R	36.0	45.4	IS							R		
III	8002L	37.0	44.1	IS		O					R		
	R	37.0	44.6	IS		P					R		
	8007L	34.0	41.7	IS				O			R		
	R	34.0	42.1	IS				P			R		
	8013L	33.0	41.6	IS		O					R		
	R	33.0	37.4	IS				P			R		
IV	8031L	31.0	38.4	IN						F			
	R	31.0	37.8	IN							R		
	8032L	32.0	39.1	IN							R		
	R	32.0	38.5	IN							R		

[a]Eye size was determined by measuring the distance from the medial canthus to the lateral canthus.
[b]Ring size is measured as the outside diameter.
[c]IS : insert ring, suture eye; IN : insert ring without suturing eye; R : remove ring and the sutures; P : partly opened eyelid; O : fully opened eyelid; F : fell out; S : resuture.

sults, there was a large variation in drug release rates, as the tearing rate was variable in these *in vivo* tests. However, one ring (VI-46-03) exhibited a nearly constant drug release rate for 5 days. Foreign material (solids, mucus, etc) from an eye resulted in a long spot trace on a developed TLC plate.

3-2. Antibiotic Susceptibility of *M. bovis*

The result of bacteriological susceptibility tests to antibiotics is shown in Table II. *M. bovis* showed a sensitive reaction to tylosin tartrate and ampicillin. Therefore, tylosin tartrate was selected to suppress the bacterium, *M. bovis* in the *in vivo* experiments.

3-3. Retention Data for Ocular Inserts in Clinical Studies

The results for the retention characteristics of the ring devices are listed in Table III. Among the groups (I, II and III) in which suturing was performed, 13 of 16 eyelids opened within 3 days after suturing. Although the sutures did not last for 7 days, most of the inserts (15 out of 16 rings) were retained until the end of the experiment (day 14). The results for the non-suture group (IV) also showed good retention characteristics for the ring devices (one for 6 days and three for more than 7 days). This demonstrated the potential to use ring devices in field applications without suturing the eyelids. Signs of undesirable side effects or eye irritation were not found. There was only a minor increase in tearing seen at the initial time of ring insertion. The shape seen for all rings after removal is shown in Fig. 1.

3-4. Quantitative Results of *M. bovis* Colonization in the Eyes

All ten calves (19 of 20 eyes) were infected with *M. bovis* after using the UV radiation eye preconditioning method. These data are shown in Table IV. Figs. 2-5 represent a plot of the logarithm of the number of colony forming units per tear swab sample as a function of time for the 14 days experiments and can be examined for changes within two time periods. The first 7-day period shows the

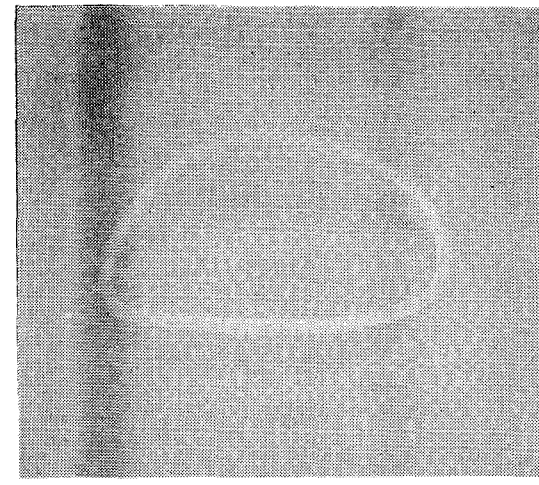

Figure 1—Typical shape of a ring device after removal from an eye. The ring size (side to side measurement) is about 50 mm.

trend in the generation and buildup (in almost all cases) of bacteria in the eye after inoculation with *M. bovis*. The second 7-day period indicates the general pattern of the change of the number of bacteria as a function of time after inserting the ring devices. Ring devices are inserted at day 7 and are removed at day 14. At day 14, the infected control eyes are treated using a fresh medicated ring (for groups I and III) or an Azimycin subconjunctival injection (for group IV) in order to compare the ring insertion method with the field method in treating the *M. bovis* infection.

In the first 7-day period, the bacteria of both eyes increased in number rapidly within 3 days. Then, the growth rate appeared to stabilize (over 10^4 CFU/swab), exhibiting only minor fluctuations. However, the bacterial growth trend for both eyes during the second 7-day period was different from that seen during the first period. That is, the number of *M. bovis* colonies of the treated eyes dropped dramatically within one or two days after the medicated rings were inserted, while the number of *M. bovis* colonies that developed from the swab sample of the control eyes maintained levels similar to that

Table IV—Number of Colony Forming Units as an Index of Changes of *M. bovis* Content in the Eye (Data expressed as $\log_{10}$ CFU/swab)

Group No.	Animal No. and eye	Days after inoculation with *M. bovis*								
		after inoculation				after treatment[c]				
		0	3	5	7	1(2)[b]	3(4)	5(6)	7(8)	8(10)
I	149L	0	4.87	4.40	3.86	0	0	1.38	1.32	0
	R	0	4.83	5.13	2.73	3.10	0.78	5.33	3.83	3.20
	150L	0	4.17	4.15	3.37	0	0	0	0	0
	R	0	0.47	4.15	3.93	3.47	3.03	0.47	0	3.20
II	152R	0	4.95	4.32	3.57	0	0	0	0	0
	L	0	3.47	3.88	4.59	5.34	5.76	5.39	2.65	0
	153R	0	4.08	5.56	4.11	0	0	0	0	0
	L	0	5.08	5.95	4.14	4.35	6.00	4.95	5.21	0
	154L	0	5.19	4.03	4.41	0	0	0	0	0
	R	0	4.38	2.96	4.50	4.73	5.03	5.89	6.08	0
III	8002R	0	4.30	4.30	3.93	0	0	0	0	0
	L[d]	0	0	0	0	0	0	0	0	0
	8007L	0	2.60	3.82	2.55	0	0	0	0	0
	R	0	3.07	2.80	4.69	4.17	5.85	6.13	5.73	0
	8013L	0	2.11	2.65	2.08	0	0	0	0	0
	R	0	4.26	3.18	2.73	5.05	5.93	4.35	5.01	0
IV	8031R	0	2.99	3.23	4.86	1.38	0	0	0	0
	L	0	3.60	3.89	4.50	5.73	4.50	3.85	3.95	1.43
	8032R	0	2.99	4.26	4.69	0	0	0	0	0
	L	0	1.95	2.26	4.50	3.65	0	2.80	0	0

[a] Both eyes of each calf were used (one as control eye and one as treated eye). First row for each calf eye number represents the data of a treated eye, while second row represents those of a control eye. L=left eye, R=right eye. [b] Data within parenthesis in this row are applied only to group I. [c] Days after treatment means the period after insertion of ring devices. [d] One eye (8002L) in group III did not show any *M. bovis*.

seen during the first 7-day period. After day 14, all treated eyes and the post-treated control eyes showed the presence of no, or only few (negligible numbers), bacterial colonies. The number of colony forming units associated with the swab tear samples provides a useful index to monitor changes taking place in the eyes in the number of *M. bovis* bacteria present for the time period covering the initial inoculation with *M. bovis* to the end of the experiment (day 17) when a swab sample was taken 2 days after removal of the medicated rings from the control eyes. The effect on the numbers during this period includes those associated with the insertion of rings (if any), the removal of rings, and the insertion of a medicated ring into control eyes.

For group I, the bacterial growth (Fig. 2) of the treated eyes (149L and 150L) was controlled by the medicated ring after day 7, while the control eye had a large number of bacteria present throughout the overall test. Two data points for the treated eye

GROUP I

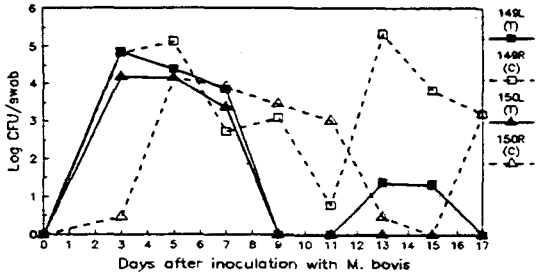

Figure 2—The bacterial growth trend with time for calf 153. Ring devices are inserted at day 7 and are removed at day 14. At day 14, the infected control eyes are treated using a fresh medicated ring.

GROUP II

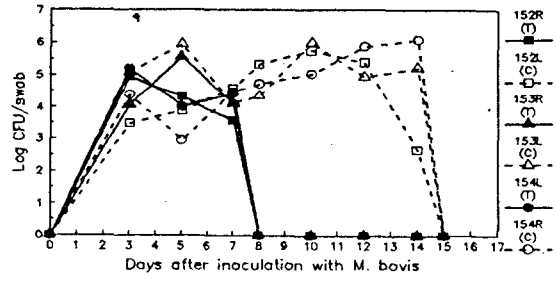

Figure 3—The bacterial growth trend with time for Group II. Ring devices are inserted at day 7 and are removed at day 14. At day 14, the infected control eyes are treated using a fresh medicated ring.

GROUP III

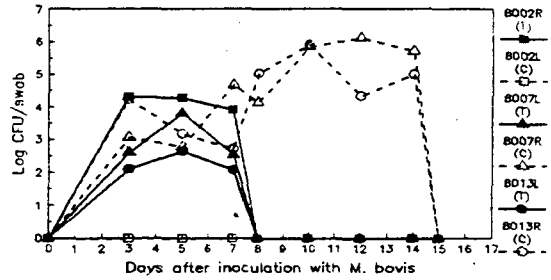

Figure 4—The bacterial growth trend with time for Group III. Ring devices are inserted at day 7 and are removed at day 14. At day 14, the infected control eyes are treated using a fresh medicated ring.

GROUP IV

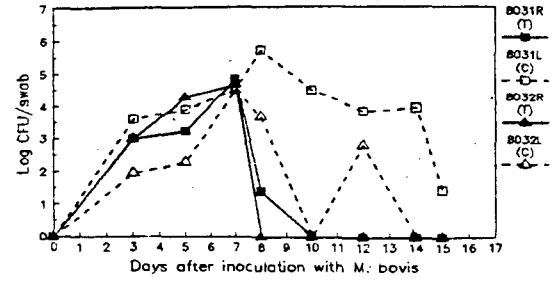

Figure 5—The bacterial growth trend with time for Group IV. Ring devices are inserted at day 7 and are removed at day 14. At day 14, the infected control eyes are treated using a fresh medicated ring.

(days 13 and 15 of 149L) showed tens of colonies even after the treatment using a medicated ocular insert; however, this amount was much less than that of the control eye (in the range of tens of thousands). Three data points from the control eyes (day 11 of 149R, and day 13 and 15 of 150R) represented a relatively low number of bacteria compared with the other data for control eyes. The eyelids were sutured during experimental period. Therefore, the discrepancy may come from a false sampling (due to sampling while the eyelid was sutured shut).

All treated eyes (152R, 153R and 154L) in group II had no *M. bovis* detectable after day 7, while the number of bacteria in control eyes (152L, 153L and 154R) remained very large for this same period in the experiment (Fig. 3). In case of 152R, the medicated ring fell out one day after insertion, but the effect of antibiotic was to eliminate the *M. bovis* within 1 day and subsequently, the infection did not become reestablished for the remained of the 7 days experiment. When a medicated ring was inserted into the control eye on day 14, the bacteria were no longer detectable (at day 15).

The left eye of calf 8002 in group III (Fig. 4) did not reproduce any *M. bovis* colonies during the entire experiment period. The right eye (treated eye) of the same animal did respond during the first 7-day period, and

after a medicated ring was inserted an immediate drop in the number of *M. bovis* occurred. In case of calves 8007 and 8013, the number of bacteria in the control eyes after day 7 increased rapidly up to $10^4 \sim 10^6$ CFU /swab, while the number of bacteria in the treated eyes were dropped to negligible levels upon application of medicated rings. At day 14, the control eyes treated with the medicated rings showed the same response as group II.

For calves 8031 and 8032 in group IV, eyelids were not sutured following ring insertion. The trend of bacterial growth (Fig. 5) for control and treated eyes was similar to group II, although at day 8 a few (negligible numbers) *M. bovis* colonies were detected in the swab sample from a treated eye (8013R). Also, note that the number of control eye (8031L) bacteria apparently decreased with time after day 8. Two data points for swab samples from the control eye (days 10 and 14 for 8032L) were zero. Ten milliliters of tetracycline were injected at day 8 to treat a severe cough for this calf. The effect of this injection can continue for 48~72 hrs after the time of injection and this may explain the control eye observations at day 10. However, it is also possible that there was a sampling problem at that time. Also, note that at day 12 of 8032L the number of colony forming units was relatively high again. Nevertheless, the medicated ring data were constant at zero colony forming units throughout the second 7-day period.

At the end of the tests, all rings were removed (day 14). Those eyes that had contained non-medicated rings then received medicted rings, and subsequent tear sampling for control eyes showed that the number of *M. bovis* colony forming units present within a day after insertion of these medicated rings became negligible.

There were differences in the degree of susceptibility of the calves to the *M. bovis* infection, and in one eye of one of the 10 calves studied, analysis of tear samples indicated that no *M. bovis* colony forming units were detectable throughout the entire test period (Table IV, calf 8002L). However, data obtained for the tear samples from the other 19 eyes clearly established the effectiveness of the medicated ring treatment.

In a comparison of the use of medicated rings in control eyes (day 14) with that of the use of a subconjunctival injection of Azimycin in a control eye (day 14), the analyses of tear samples from both of these two types of treatments showed that the numbers of *M. bovis* colony forming units dropped to negligible levels (group IV animals).

3-5. Clinical Examination

After the inoculation of *M. bovis*, clinical signs for the infected eyes changed to more severe stages (central corneal perforation or scar). Some eyes did not show clinical signs of IBK, even though *M. bovis* colonies were found in certain infected eyes. After applying ring devices, the severe clinical signs of the medicated eyes changed to the normal stage or healing process stage. However, the clinical signs for non-medicated eyes continued to gradually worsen with time. The change of clinical signs with time is listed in Table V. Clinically, 7 of 20 eyes showed mild or more severe signs of infection due to *M. bovis* (Table V). Most of the eyes showing the more severe signs were used as treated eyes (i.e., they received a medicated ring) in order to evaluate the effect of the medicated ring device on IBK infections. After applying ring devices, the mild or more severe clinical signs of the medicated eyes changed to the normal stage or the healing process state (after Jackson, 1953). For the eyes receiving medicated rings, two of the 10 eyes exhibited no signs of IBK throughout the treatment period. Eight of the 10 eyes showed improvement. Of these 8 that showed improvement, 4 changed back to what appeared to be a normal condition (i.e., no signs of IBK). However, the clinical signs for 2 out of 10

Table V—Clinical Signs : Medicated and Non-medicated Devices

Before ring insertion			After ring insertion		
Clinical signs		No. of cases	Applied ring type	Clinical signs[a]	No. of cases
day 0	day 7			day 7 day 14	
Normal	to normal	8	Non-medicated	Normal to normal	6
			Medicated	Normal to normal	2
Normal	to initial	5	Non-medicated	Initial to mild	1
			Non-medicated	Initial to normal	2
			Medicated	Initial to normal	2
Normal	to mild	2	Non-medicated	Mild to more severe	1
			Medicated	Mild to healing	1
Normal	to more severse	5	Medicated	More severe to normal healing	2 3
Total		20		Total	20

[a] This is defined as the follows : normal sign-no change on the eye; initial-photophobia, epiphora, and blepharospasm; mild-conjunctivitis or keratitis; more severse-central corneal ulceration; healing-vascularization of cornea (after Jackson, 1953; Punch and Slatter, 1984).

non-medicated eyes continued to gradually worsen with time.

4. DISCUSSION

Tear samples were collected from the eyes of healthy cows by using cotton swabs. The tears contained tylosin tartrate released from medicated rings in the eyes. The antibiotic release rate was characterized using a multiple spotting TLC technique. Most of the spots of tear samples collected from healthy cows were detectable. However, some traces on the plates were elongated, and this complicated the TLC analysis. This was probably due to naturally occurring contaminants in the tears. The drug release rates measured in *in vivo* experiments were lower than those seen from *in vitro* tests (Ryoo, 1986). This might result from the variable tearing rate seen during the *in vivo* tests. All of the ring devices studied released drug amounts above the minimum inhibitory concentration of tylosin (1.2 μg/m*l*) necessary for treating IBK.

M. bovis was sensitive to tylosin tartrate in the antibiotic susceptibility test. The effectiveness of tylosin tartrate as the treatment drug can be seen in Figs. 2-5. Within one day after inserting a ring device containing tylosin tartrate, the number of *Moraxella bovis* colonies was rapidly reduced to negligible levels.

In order to reproduce the disease (pinkeye) in the eyes of healthy calves, the ultraviolet radiation preconditioning method was used. Ninety five percent of the eyes (19 of 20 eyes) of infection studies reproduced *M. bovis* in the eyes. Although initially suturing of eyelids was performed in order to prevent the ring devices from falling out, the sutures usually pulled out within 3 days. Additional tests showed that this technique was not necessary in order to retain the ring devices in the eye for 7 days. Four rings of Group IV showed good retention characteristics without the use of sutures.

Results (Table IV) of calf groups I, II, III and IV indicated that at day 7 of the infection and treatment experiment almost all of the calf eyes contained large numbers of *M. bovis* before the treatment of inserting a medicated ring into one eye and a non-medicated ring

(a control) into the other eye of each calf. The number of colony forming units found in swab samples of tears from the eyes were seen to decrease dramatically within a day after insertion of medicated rings. By taking these samples during the 7-day period following insertion of the rings, an index of changes occurring in the eyes during the insertion period was obtained.

At the end of the tests, all rings were removed (day 14). Those eyes that had contained non-medicated rings then received medicated rings, and subsequent tear sampling for control eyes showed that the number of *M. bovis* colony forming unts present within a day after insertion of these medicated rings became negligible.

There were differences in the degree of susceptibility of the calves to the *M. bovis* infection, and in one eye of one of the 10 calves studied, analysis of tear samples indicated that no *M. bovis* colony forming units were detectable throughout the entire test period (Table IV, calf 8002L). However, data obtained for the tear samples from the other 19 eyes clearly established the effectiveness of the medicated ring treatment.

Sixty percent of the eyes in the infection studies (12 out of 20) showed clinical signs of IBK. Four out of these 12 which showed clinical signs of IBK received non-medicated rings, and two of these four eyes continued to worsen gradually with time. Of the eyes receving medicated rings and showing signs of IBK, 50% exhibited significant improvement (4 of 8) in that no apparent signs of IBK were observed at the end of the treatment period. There are 2 cases in which corneal lesions were present at the time of insertion of medicated rings. Seven days later when the medicated rings were removed, the eyes appeared to be normal. The other 50% were in healing stages; however, it is not clear if the medicated rings played a major role in these cases. However, 20% of the eyes receiving non-medicated rings (2 of 10) continued to worsen gradually with time. Direct bacterial counts and clinical observations clearly showed that a sufficient amount of antibiotic was released to treat IBK or to provide therapeutic protection.

5. CONCLUSIONS

Ten calves were tested to evaluate the effectiveness of medicated hydrogel ocular inserts for treating pinkeye in cattle. An ultrabiolet radiation method followed by instillation of *M. bovis* reproduced IBK in 19 out of 20 eyes. The severity of the ocular infection varied from a normal stage to a more severe stage (corneal ulceration). *M. bovis* was sensitive to tylosin tartrate. Medicated rings were very effective in suppressing the growth of bacteria within 1 to 2 days. Clinically, most of treated eyes improved from a severe stage to a normal or healing stage. However, control eyes (receiving non-medicated rings) changed to more severe stages with time. The infection was also monitored by sampling bacteria from the eyes, and noting the change in the number of colony forming units found from the tear solutions in the various swab samples taken at different times and conditions (i.e., before and after medicated rings were used). Upon application of the medicated rings, usually within a day the bacterial numbers had decreased to negligible levels and remained very low or nondetectable throughout a 7-day period after insertion. Initially, eyelids were sutured together to insure that the rings would remain in place throughout an experiment; however, it was found that suturing was not necessary. The ring devices are very useful in treating IBK, and can be utilized easily and effectively in the field. The devices release suitable amounts of drug continuously, are easy to insert and to retain, and are inexpensive.

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