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RESEARCH ARTICLE

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The Treatment of Infectious Bovine Keratoconjunctivitis Under Field Conditions: Intrapalpebral Injection Versus Subconjunctival Injection of Oxytetracycline

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ABSTRACT

The purpose of this study was to determine the efficacy of oxytetracycline when applied intrapalpebral (IPa) injection to the eye, compared with subconjunctival (SCo) injection, in the treatment of infectious bovine keratoconjunctivitis (IBK). Twenty eyes with severe clinical signs of IBK, of 15 Holstein Friesian female cattle aged 2–4 years were evaluated in this study. The affected animals had unilateral (n=10; 66.7%) and bilateral (n=5; 33.3%) symptoms. *Moraxella bovis* were identified in all ocular swabs samples on day 0 and all isolates were susceptible to oxytetracycline (100%). Oxytetracycline (100 mg/ml) was injected IPa (n=10) and SCo (n=10) at a total dose of 200 mg once daily on days 0, 3 and 6. After injections, the animals were re-examined for resolution of lesions associated with IBK weekly until the corneal ulcer healed. Microbiologic examination was repeated 3 times at intervals of 1 week. There is no effect of injection type on healing time of the lesion. Size and side of the lesion have a significant effect on healing time ($p<0.001$). It was found that small lesions had earlier clean in terms of the microbiologic evaluation. Lesion size has a significant effect on microbial growth ($p<0.001$). In conclusion, the same therapeutic effect was achieved in both applications. However, oxytetracycline given by IPa injection was comparatively easy, more comfortable and less invasive especially for painful eyes against IBK than SCo injection under the conditions of this study. In addition, it was enough for cattle with IBK at the dosage (200 mg, once daily on days 0, 3 and 6) of used in the study.
Keywords: cattle, IBK, intrapalpebral, oxytetracycline

Saha Koşullarında Enfeksiyöz Bovine Keratokonjuktivitis'in Sağaltımı: Subkonjuktival Oksitetrasiklin Enjeksiyonuna Karşı İntrapalpebral Enjeksiyon

ÖZ

Bu çalışmanın amacı, infeksiyöz bovine keratokonjuktivitis (IBK)'in sağaltımında, göze intrapalpebral (IPa) enjeksiyon ile uygulanılan oksitetrasiklin'in, subkonjuktival (SCo) enjeksiyon ile karşılaştırıldığındaki etkinliğini belirlemekti. Çalışmada, IBK'nın şiddetli klinik bulguları bulunan 2-4 yaşındaki, dişi, 15 Holstein Friesian sığıra ait 20 göz değerlendirildi. Etkilenen hayvanlar unilateral (n=10; % 66,7) ve bilateral (n=5; % 33,3) semptomlara sahipti. 0. günde tüm oküler swab örneklerinden *Morexella bovis* izole edildi ve oksitetrasikline duyarlı idi (% 100). 0, 3 ve 6. günlerde günde bir kez toplam 200 mg dozda oksitetrasiklin (100 mg/ml) IPa (n=10) ve SCo (n=10) olarak enjekte edildi. Enjeksiyonlardan sonra, korneal ülser iyileşmesi şekilleninceye kadar haftalık olarak IBK ile ilişkili lezyonlar yönünden hayvanlar tekrar muayene edildi. Mikrobiyolojik muayene birer hafta ara ile 3 kez tekrarlandı. Lezyonun iyileşme zamanı üzerine enjeksiyon tipinin etkisi yoktu. Lezyonun büyüklüğü ve bulunduğu tarafın iyileşme zamanı üzerine belirgin etkisi vardı ($p<0.001$). Küçük lezyonlar mikrobiyolojik değerlendirmede daha erken dönemde temiz bulundu. Lezyon büyüklüğü mikrobiyal gelişimi belirgin olarak etkiledi ($p<0.001$). Sonuç olarak, her iki uygulama ile aynı terapötik etki sağlandı. Bununla birlikte, bu çalışmada saha koşullarında özellikle ağrılı gözlerde IPa enjeksiyon yolu ile verilen oksitetrasiklin, SCo enjeksiyondan nispeten daha kolay, daha rahat ve daha az invazivdi. İlave olarak, bu çalışmada kullanılan doz (0, 3 ve 6. günlerde günde bir kez 200 mg) IBK'lı sığırlar için yeterli idi.
Anahtar kelimeler: IBK, intrapalpebral, oksitetrasiklin, sığır

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INTRODUCTION

Infectious bovine keratoconjunctivitis (IBK) is a highly contagious ocular disease and spreads rapidly among cattle in a herd (Alexander 2010, Angelos et al. 2001). *Moraxella bovis* (*M. bovis*) is considered the primary causal organism associated with IBK (Bedford 2004). However, the recently characterized Moraxella bovoculi (*Mor bovoculi*) has been isolated from eyes of calves and cattle affected with IBK. *Moraxella bovis* and *Mor bovoculi* are generally considered to be susceptible to a variety of different antibiotics (Angelos 2015). IBK treatment trials had been published in the ensuing decade that would enable better understanding of comparative efficacy of antibiotic treatment options for IBK (Cullen 2016). Numerous antibiotic drugs including penicillin, gentamicin, neomycin–bacitracin–polymyxin B, sulfonamides, oxytetracyclines, furazolidone, clindamycin, chloramphenicol, florfenicol and ceftiofur have been used in the treatment of IBK with systemic and topical applications, with variable results (Burns and O'Connor 2008). Long-acting intramuscular formulation of oxytetracycline is commonly used in cattle to treat infected with IBK. The main disadvantage of using the intramuscular route for treatments is the large amount of drug that has to be administered (Zielinski et al. 2002).
To achieve therapeutic drug concentration, topical antibiotic administration as solutions, powders, or ointments are required several times per day (Brown et al. 1998, McConnel et al. 2007). But the major disadvantage of topical treatment is the short duration of action of the active ingredient in ocular tissues due to the limited lacrimal fluid and low levels of drug achieved in the ocular tissue (Zielinski et al. 2002). Moreover, the daily multi-dose therapy is usually not practical for most farmers (Brown et al. 1998, McConnel et al. 2007).
Subconjuctival injections (SCo) was also used for the topically administration of antibiotics. Subconjunctival treatment requires only a small amount of drug and achieves high antibiotic levels in lacrimal fluid and ocular tissue, however, application is very difficult in fractious and painful animals and can cause ocular tissue damage (Zielinski et al. 2002). Different applications are required for cattle which have similar therapeutic efficacy with SCo but less invasive and easier to administer in field condition. In the literature, the application of tilmicosin by intrapalpebral injection (IPa) was also found effective but there is insufficient data to support this route of administration (Zielinski et al. 2002).

The purpose of this study was to determine the clinical efficacy of IPa oxytetracycline against naturally occurring cases of IBK and to compare this efficacy with a SCo dose of oxytetracycline under field conditions.

MATERIALS and METHODS

The study was conducted during the October and November at a dairy herd in Bursa, Turkey. Twenty eyes (n=20) with severe clinical signs of IBK, of 15 Holstein Friesian female cattle aged 2–4 years were evaluated in this study. The affected animals were separated from the herd. The ocular conjunctival swabs were obtained from the each animal's conjunctival sac of eyes using sterile cotton-tipped swabs for isolation of *M. bovis*. These samples were placed in screw-capped tubes containing sterile Stuart transport medium (Oxoid) and transported to the laboratory. Swabs were streaked on 10% sheep blood agar plates which were then incubated at 37 °C for 48 to72 h under aerobic conditions. *M. bovis* was isolated and identified by using standard microbiologic techniques (George et al. 1984). Antibiotic sensitivity testing was performed using the disk diffusion method (George et al. 1985).

Oxytetracycline (100 mg/ml; Primamycin®, Pfizer) was injected intrapalpebrally (IPa, n=10) and subconjunctivally (SCo, n=10) at a total dose of 200 mg (2 ml), once daily on days 0, 3 and 6. During the IPa injections, the animal's head simply hold and the upper eyelid skin was lifted to provide an acute angle with respect to the eye's surface, and a 21 gauge needle was inserted into the base of the fold created and antibiotic injection was made (Figure 1A). During the SCo applications, the animal's head was well restrained, the upper eyelid was rolled back, and a 25 gauge needle was inserted into the dorsal palpebral conjunctiva and antibiotic was injected (Figure 1B). After injections, the animals were re-examined for resolution of lesions associated with IBK weekly until the corneal ulcer healed. Corneal ulcers were photographed. Photographic images were recorded by a Kodak EasyShare DX4530 camera. Before photos were taken, a ruler with clear millimeter divisions was placed near the lower eyelid. Care was taken to ensure that the camera was angled perpendicular to the cornea.

The animals were assessed for 28 days after treatment for 8 clinical signs of infection and presented in Table 1. Microbiologic examination was repeated 3 times at intervals of 1 week (on days 7, 14 and 21).

Statistical analyze

To test the effects of injection type, size and side (unilateral or bilateral) of the lesion on healing time, three-way anova (analysis of variance) was conducted using GLM procedures. P values are set ≤.05 to be considered significant. The results are shown as means ± standard errors of the mean (SEM) (Table 2).

RESULTS

M. bovis was detected in all samples on day 0. All isolates were sensitive to oxytetracycline (100%), enrofloxacin, gentamicin, clindamycin and penicillin G, respectively. In animals, bacteriologic cultures were negative at the end of treatment (Table 1).

Table 1. Clinical and laboratory evaluation on day 0 and post treatment period (on days 7, 14, 21 and 28)
Tablo 1. 0. gün ve sağaltım sonrası dönemde klinik ve laboratuar değerlendirmeleri (7, 14, 21 ve 28. günler)

Oxytetracycline applications	Days	M.bovis isolation*	Swelling lids	Blepharospasm	Epiphora	Photophobia	Keratitis	Corneal ulcer**	Corneal stroma***	Partial loss of view
Subconjunctival injection group (SCo, n=10)	***0***	10	+	+	+	+	+	+	+	+
	7	8	↓	-	-	-	↓	↔	↓	↔
	14	5	-	-	-	-	↓	↓	↓	↓
	21	0	-	-	-	-	↓	↓	-	-
	28	-	-	-	-	-	-	-****	-	-
Intrapalpebral Injection group (IPa, n=10)	***0***	10	+	+	+	+	+	+	+	+
	7	8	↑	↓	-	-	↓	↔	↓	↔
	14	6	↓	-	-	-	↓	↓	↓	↓
	21	0	-	-	-	-	↓	↓	-	-
	28	-	-	-	-	-	-	-****	-	-

*: The number of cattle *M. bovis* isolated from ocular secretions, **: Ø ≥ 0.5 cm, ***: Common white to deep yellow opacity in the Corneal stroma, ****: Four animals recovered with permanent slight scarred cornea, +: yes, -: no, ↑: increased, ↓: decreased, ↔: stable

Table 2. The effects of injection type, size and side of the lesion on healing time (HT) and microbial growth (MG)
Tablo 2. İyileşme zamanı ve mikrobiyal gelişme (MG) üzerine lezyon büyüklüğünün, bulunduğu tarafın ve enjeksiyon tipinin etkileri

	Injection type (IT)			Lesion size (LSi)			Lesion side (LSd)		
	SCo (n= 10)	IPa (n=10)	P value	Small (n=4)	Large (n=16)	P value	Unilateral (n=10)	Bilateral n=10	P value
HT (day)	20.2±0.78	21.0±0.72	NS	14.0±1.08	23.9±0.58	**<0.001**	17.5±0.71	26.8±0.70	**<0.001**
MG (week)	2.01±0.16	2.17±0.15	NS	1.00±0.23	2.67±0.12	**<0.001**	1.75±0.15	2.83±0.15	NS
P Interactions of HT									
IT x LSi					NS				
IT x LSd					NS				
LSi x LSd					NS				
IT x LSi x LSd					NS				
P Interactions of MG									
IT x LSi					NS				
IT x LSd					NS				
LSi x LSd					NS				
IT x LSi x LSd					NS				

NS: Not significant

The affected animals had unilateral (n=10; 66.7%) and bilateral (n=5; 33.3%) symptoms. Prior to treatment, clinical signs of IBK generally included swelling of the lids, ocular discharge, blepharospasm, neovascularization and corneal ulcer diameter greater than 0.5 cm, a common white to deep yellow opacity in the corneal stroma (Figure 2A-D).

Corneal ulcer diameter were measured (cm) on picture and the values were found 1.35 ± 0.36 in SCo and 1.41 ± 0.48 in IPa group (Mean ± SD). Corneal opacity was clearly reduced in all cases which was noticed during the second injection. A painful swelling of the upper eyelid was occurred in all animals of IPa group after the second injection of oxytetracycline. This swelling disappeared by 72 hours after injection.

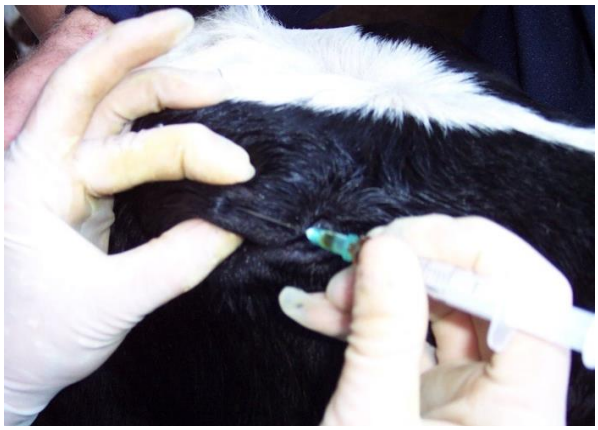

Figure 1A

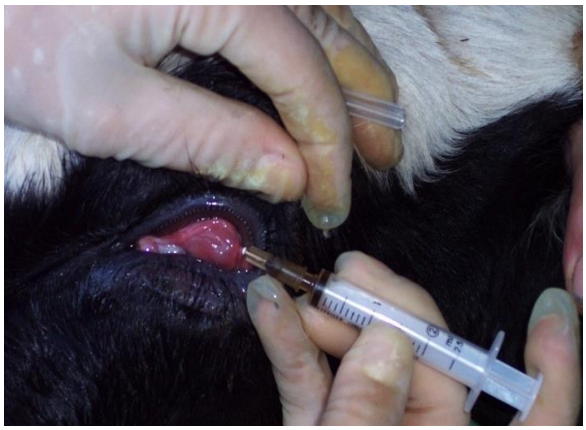

Figure 1B

Figure 1. Application of different oxytetracycline injection techniques in IBK cases. A. Intrapalpebral (IPa) injection, B. Subconjuktival (SCo) injection.

Şekil 1. IBK vakalarında farklı enjeksiyon tekniklerinin uygulanması. A. İntrapalpebral (IPa) enjeksiyon, B. Subkonjuktival (SCo) enjeksiyon.

Figure 2A

Figure 2B

Figure 2C

Figure 2D

Figure 2. A bluish-white opacity of the surface of the Cornea (A), deep corneal ulceration (B), a common white to deep yellow opacity in the corneal stroma and neovascularization (C) and swelling of the lids (D).

Şekil 2. Kornea üzerinde mavimsi beyaz opasite görünümü (A), derin korneal ülserasyon (B), korneal stromada sarımsı-beyaz yaygın opasite ve neovaskülarizasyon (C) ve göz kapaklarında şişlik (D).

Corneal ulcers recovered from day 14 (n=4), day 21 (n=8) and day 28 (n=8). In both groups 2 animals (2 eyes in 2 animals from group 1, and 2 eyes in 2 animals from group 2) were recovered with a permanent slight scarred cornea. There is no effect of injection type on healing time of the lesion ($p=0.408$). Size and side of the lesion have a

significant effect on healing time ($p<0.001$). The cattle with small ocular lesion had faster healing time (in 14 days) than that of the cattle with large ocular lesion (between 21 and 28 days). The lesions occurring one side led to faster healing time. In the microbiologic evaluation, small lesions were earlier clean (pathogen free) than large lesions. Lesion size has a significant effect on microbial growth ($p<0.001$). There were no interactions between injection type, lesion size and lesion side ($p>0.05$).

DISCUSSION

Subconjunctivally administered medications usually maintain higher corneal drug concentrations for longer periods of time than topical or parenteral applications (Senturk et al. 2007, Alexander 2010). In this application, the antibiotic must be placed beneath the dorsal palpebral or bulbar conjunctiva but it can be difficult to achieve in fractious cattle and requires good restraint (Quinn et al., 1994). It is very important that the animals should be properly restrained and fixation of the animal's head during SCo injection. This causes time and labour loss. On the other hand, palpebra is very mobile and pliable compared with skin elsewhere; therefore, the drugs will be injected more easily intrapalpebrally. During the literature search could not be reached adequate information about the advantages or disadvantages of IPa injection for treatment of IBK. There is no comparative study of SCo and IPa injection techniques. Researchers usually studied either the effects of different drugs or the effects of different doses of the same drug in cattle with IBK (George et al. 1985, Gokce et al., 2002, Zielinski et al. 2002, Senturk et al. 2007). In the present study, two injection techniques were compared. The IPa injection technique allowed easy application even in fractious animals and the drug was also injected more easily. Clinically the healing time was found similar in both groups. It was definitely a more comfortable application than SCo injection in field conditions and provided the same therapeutic effect.

It has been demonstrated that *M. bovis* is susceptibility to a variety of antibiotics (Prieto et al. 2013) and appropriate antimicrobial selection for the treatment of cattle infected with *M. bovis* requires knowledge of the minimum inhibitory concentration (MIC) for the bacterium, as well as an understanding of antibiotic distribution into ocular tissues and tears following administration (Shryock et al. 1998). The oxytetracycline is usually the first choice for antimicrobial treatment of IBK (Pickett 1999, Gokce et al., 2002, Senturk et al. 2007). Researchers have reported that the concentrations of the oxytetracycline-LA formulation might be administered for treatment of bacterial diseases in 2 days intervals but systemic administration of the drug may not ensure an effective therapy in eye disease (Zielinski et al. 2000, Zielinski et al. 2002). Maintaining consistent therapeutic drug concentration in tear film is difficult because of practical consideration. It has been reported to achieve therapeutic drug concentration, topical antibiotic administration is required several times per day; however, daily multidose therapy is not practical for most producers (Pickett 1999). Subcutaneous, intramuscular, and intravenous antibiotics are commonly used but very high dosages of an antibiotic are required to ensure adequate levels of the drug reach the eyes and tear glands (Brown et al. 1998, McConnel et al. 2007). Researchers have reported that the SCo administration of oxytetracyclineis reduce total dosages of drug than administration of systemic and daily multidose topical therapies (McConnel et al. 2007). High concentrations of antibiotic may be achieved in tear film (for 72 h) by SCo administration, although local irritation may occur (Brown et al. 1998). SCo injection of tetracyclines is effective but may cause necrosis at the injection site (Brown et al. 1998, Pickett 1999). Additionally, oxytetracycline-LA should not be recommended as a subconjunctivally, due to the it's severely irritation (Pickett 1999, Alexander 2010). The present study, all isolates were susceptible to oxytetracycline (100%) and conventional formulation of oxytetracycline preferred instead of oxytetracycline-LA for treating IBK by SCo and IPa routes. Only a painful swelling of the upper eyelid was occured in all animal of IPa injection group after the second injection of oxytetracycline, but there was no finding of necrosis in injection site at the end of treatment.

In the present study, all active symptoms such as blepharospasm, epiphora, photophobia were almost resolved at the end of 7 days in both groups, except of corneal ulcers (28 days). According to the findings and observations during this study, IPa injections ensure an effective therapy of the corneal ulcers caused by IBK in cattle. Additionally, it could be considered that, clinical findings of IBK rapidly and similarly resolved as in SCo injections. Based on literature knowledge (Brown et al. 1998, Zielinski et al. 2002)

in the present study these clinical results may be interpreted as the lacrimal fluid and ocular tissues contain sufficient quantity of oxytetracycline for a long time in both treatment group. In addition, it is safe for cattle with IBK at the dosage and the frequency of application of oxytetracycline used in this study. Since it was beyond of the scope of this study, the concentration of the oxytetracycline in the lacrimal fluid was not evaluated. The search of the amount of oxytetracycline in the lacrimal fluid after both local injections (SCo and IPa) during IBK treatment would be evaluated in the further studies.

CONCLUSION

In conclusion, 3 days intervals (days 0, 3 and 6), 3 IPa application of conventional formulation of oxytetracycline appears to be an effective method for the treatment of IBK with severe clinical symptoms such as corneal ulcers in this study, and IPa injection was recommended due to the easier application in field conditions. In addition, it was enough for cattle with IBK at the dosage (200 mg, once daily) used in the study.

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