Original Article

pISSN 2466-1384 · eISSN 2466-1392
Korean J Vet Res 2021;61(4):e37
<https://doi.org/10.14405/kjvr.2021.61.e37>

*Corresponding author:

Taeho Oh
Department of Veterinary Internal Medicine,
College of Veterinary Medicine, Kyungpook
National University, 80 Daehak-ro, Buk-gu,
Daegu 41556, Korea
Tel: +82-53-950-5959
Fax: +82-53-950-7488
E-mail: thoh@knu.ac.kr

ORCID:
<https://orcid.org/0000-0003-2898-6476>

Conflict of interest:
The authors declare no conflict of interest.

Received: July 12, 2021
Revised: September 17, 2021
Accepted: October 14, 2021

© 2021 The Korean Society of Veterinary Science.
This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial license (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

In vivo and *in vitro* efficacy of florfenicol, terbinafine, and mometasone furoate topical otic solution for the treatment of canine otitis externa

Seulgi Bae, Yunseok Jin, Taeho Oh*

Department of Veterinary Internal Medicine, College of Veterinary Medicine, Kyungpook National University, Daegu 41556, Korea

This study evaluated the efficacy of a new, single-dose otic solution combining florfenicol, terbinafine, and mometasone furoate for the treatment of canine otitis externa (OE) *in vitro* and *in vivo*. Forty-one client-owned dogs with OE were included in the study and divided into a treatment group that received the test solution and a negative control group that received a normal saline solution. On day 0, the dogs were treated either with the test or the control solution and evaluated over 30 days. Clinical efficacy was evaluated by clinical signs and cytological organism counts. *In vitro* antimicrobial susceptibility was evaluated by the minimum inhibitory concentration (MIC). After treatment with the test solution, clinical signs continuously decreased and cytological scores were significantly reduced. The results of MIC testing showed that the test solution was potent against the common pathogenic causes of canine OE. In this study, the most common causative pathogens were *Staphylococcus pseudintermedius*, *Pseudomonas* spp. and *Malassezia pachydermatis*. No issues related to safety were identified. Based on these results, this new ototopical drug can be used as first line treatment for canine OE.

Keywords: dogs; otitis externa; florfenicol; terbinafine; mometasone furoate

Introduction

Canine otitis externa (OE) is one of the most common dermatological problems for which dog owners frequently seek veterinary care and is associated with a high rate of recurrence [1-3]. Dogs with OE may show erythema, swelling, exudate, and clinical signs related to pruritus and pain due to inflammation in the external ear canal [3,4].

Numerous underlying issues can cause or worsen OE; however, addressing pathogens such as bacteria or yeast plays an important role in the management of OE. Although various pathogens are associated with canine OE, *Staphylococcus pseudintermedius*, *Pseudomonas aeruginosa*, and *Malassezia pachydermatis* are the most common pathogens found in this condition [3-7]. Thus, reducing number of these infectious organisms is critical to the successful treatment of OE. For this purpose, topical and systemic antimicrobials with or without anti-inflammatory agents are used in veterinary clinics [3].

The otic solution used in this study (Neptra; Elanco, USA) includes florfenicol, terbinafine, and mometasone furoate in a single-dose regimen. This otic solution is the Food and Drug Administration–approved, veterinarian–administered treatment for canine OE, and *S. pseudintermedius* and *M. pachydermatis* show suscep-

tibility to the active ingredient. Similar to chloramphenicol, florfenicol is a broad-spectrum antibiotic with efficacy against many bacteria. This drug was investigated for use in treating respiratory diseases in domestic animals as well as dermatologic diseases in small animals [8-10]. Terbinafine is an antifungal agent used primarily for dermatophytosis but may be useful for *Malassezia* spp. dermatitis [11-13]. Finally, mometasone furoate is a highly potent topical corticosteroid used to treat pruritic and/or inflammatory conditions in skin infection [14]. A previous study demonstrated the efficacy of this formulation in the treatment of OE [15]. In addition, the active ingredients in this solution are not generally used in Korea, thus we explored the merits of this otic solution on canine OE in our country.

The purpose of this study was to evaluate the *in vivo* and *in vitro* efficacy of an otic solution containing florfenicol, terbinafine, and mometasone furoate for the treatment of canine OE.

Materials and Methods

Animals

Dogs diagnosed with OE caused by bacterial and fungal infection with any clinical signs were included. Inclusion criteria further included a minimum age of 12 weeks and intact tympanic membrane. Animals with any concurrent ear disease associated with OE (e.g., neoplasia, polyp, or parasite otitis) or with any systemic disease requiring treatment, such as immune-mediated or hormonal disease, were excluded. Dogs administered with anti-infective, and antifungal and anti-inflammatory agents during the last 4 weeks prior to the study were also excluded.

Study design

Before the study, the experiment process was briefed to the dog's owners, and they consented to their dogs' inclusion in the study. The animals were randomly divided into the group that received the test solution or the other group that received a saline solution as negative control. Prior to the application of each solution, ear cleaning was performed using warm saline solution. The dosage for both, the test and control solution, was 1 mL per one ear.

The animals were observed on day 0, day 7 $\pm$ 2, day 14 $\pm$ 2, and day 30 $\pm$ 2. The overall schedule of events is shown in Table 1. Throughout the study clinical examination and scoring were performed using otoscopic examination and total clinical scoring (TCS) system. The TCS system evaluated for erythema, swelling, erosion/ulceration, and exudate based on severity scores ranging from 0 (absent) to 3 (severe). The primary end-

point of the efficacy evaluation was expressed as success or failure of the treatment. If the TCS score was less than 3 points on the last visit day and the scores for each evaluation factor of TCS did not deteriorate, the treatment was judged as a "success".

At the initial and final visits, ear swabs for pathogen culture and cytology were conducted. Each ear exudate sample was smeared onto a clean slide and stained with Diff-Quik. The clinician scanned the slide and evaluated infectious organisms at $\times 1,000$ magnification (high power field). Five to ten high power fields were scanned in each evaluation, and average counting number of infectious organisms were used for scoring. The scoring criteria for bacteria and yeast count on cytology are shown in Table 2 [16]. The secondary endpoint of the efficacy evaluation was also expressed as "success" or "failure" of the treatment. If the cytology scores per bacteria or *Malassezia* decreased more than 2 points on day 30 compared with day 0, the treatment was judged as successful.

Microbiological culture and minimum inhibitory concentrations tests

Ear exudate samples were cultured in 5% sheep blood agar and MacConkey agar at 37°C for 24 hours and Sabouraud dextrose agar at 35°C for 24 to 48 hours aerobically. The identification of organisms was done by polymerase chain reaction. The minimum inhibitory concentration (MIC) results were interpreted as the lowest concentration of antimicrobial agent that

Table 1. Schedule of experiment

	Day 0	Day 7	Day 14	Day 30
Physical examination	○	○	○	○
Ear swab for culture and cytology	○			○
Otoscopic evaluation	○	○	○	○
Administration of solution	○			
Recording of adverse event	○	○	○	○

Table 2. Semi-quantitative scale for cytological organism counts

Organism	Count/high power field	Score
<i>Malassezia</i> yeasts	0	0
	1-2	1 (+)
	3-8	2 (++)
	> 8	3 (+++)
Cocci/rods	0-2	0
	3-8	1 (+)
	9-40	2 (++)
	> 40	3 (+++)

Five to ten high power fields were observed in each counting, and average counts were used for scoring.

completely inhibited the growth of the organism. The isolated bacteria and yeast specimens were tested for MIC against florfenicol and terbinafine, respectively. The testing was conducted by microdilution as recommended by the Clinical and Laboratory Standards Institute [17,18].

Safety evaluation

At each of the visits, the investigator conducted a physical examination for the evaluation of the safety of the test solution. In addition, the investigator educated the owner to contact or visit an animal hospital in the case of an adverse event such as for example head tilt, nystagmus, or circling during the study period.

Statistical analysis

The experimental unit was the individual dog. In bilateral OE, both ears were treated, but the ear with the higher entry score was selected as the study ear. In the case of equal scores, the right ear was determined to be the study ear. All analyses were performed with GraphPad Prism version 9.0.0 for Windows (GraphPad Software Inc., USA). The difference in treatment success rates between the 2 groups was evaluated using Fisher exact test at a significance level of 0.05 or less. Quantitative analyses for TCS and cytological evaluation were performed using Wilcoxon code ranking test and Mann-Whitney U-test.

Results

Animals

A total of 41 dogs were included in the study with 28 subjects receiving the test solution and 13 the control solution. Two subjects in the treatment group dropped out because of owner non-compliance. Both subjects did not undergo efficacy evaluation, but MIC was performed from exudate collected from one of them. During the clinical trial period, the subjects were kept in their usual living conditions. The most common breed was the Maltese (26.8%, 11/41), followed by the Poodle (14.6%, 6/41). The mean age was 7 years (range, 1 to 18 years). Animal experiments were performed according to the Guide for the Institutional Animal Care and Use Committee of the Kyungpook

National University (IACUC-2019-0117).

Clinical efficacy

TCS evaluation

The average TCS scores on day 0, day 7, day 14, and day 30 of each group are shown in Table 3 and Fig. 1. The mean TCS on day 0 of the treatment and control groups were 6.27 and 5.62, respectively and no significant difference in the mean TCS was found between the groups. On day 14, the mean TCS of the treatment group was 2.42, which was lower than that of the control group at 4.46. On day 30, the mean TCS of the treatment group decreased to 1.85; however, that of the control group on day 30 increased to 4.54. The differences in the mean TCS between treatment and control group were statistically significant on day 14 and day 30 ($p < 0.01$ and $p < 0.001$, respectively). The treatment success rate based on TCS (primary efficacy endpoint) was 88.5% (23/26) in the treatment group and 7.7% (1/13) in the control group.

Cytological evaluation

Cytological evaluation using organism counts was conducted

Fig. 1. Comparison of average total clinical scores on day 0, day 14, and day 30 of treatment group and control group. * $p < 0.01$, $^+p < 0.001$.

Table 3. Average total clinical scores on days 0, 7, 14, and 30

TCS	Number	Day 0	Day 7	Day 14	Day 30
Treatment group	26	6.27 ± 1.46	3.23 ± 1.86	2.42 ± 1.84	1.85 ± 1.71
Control group	13	5.62 ± 0.96	4.31 ± 2.53	4.46 ± 1.51	4.54 ± 1.66

Values are presented as mean ± SD unless otherwise indicated.

on day 0 and day 30. Table 4 shows the success rates of bacterial and yeast cytology score reductions based on the secondary endpoint in the treatment and control groups. A significant reduction in organism count was achieved in the treatment group ($p < 0.001$).

Secondary endpoint efficacy evaluation findings are shown in Table 4. The success rate of bacterial reduction was 88.5% and 23.1% in the treatment and control group, respectively. The success rate of yeast reduction was 96.2% and 23.1% in the treatment and control groups respectively. These results indicate a statistically significant difference in the reduction of organism count in the treatment group ($p < 0.001$).

Microbiological culture and MIC tests

A total of 54 bacteria and 50 fungi were isolated, and subsequent MIC evaluation was conducted on the pathogens (Table 5). Florfenicol showed an inhibitory effect on the growth of *S. pseudintermedius* from concentrations of 4 to 16 µg/mL *in vitro*. The MIC ranges of *P. aeruginosa* and *Staphylococcus schleiferi* were observed at florfenicol concentrations of 2 to 16 µg/mL and 2 to 8 µg/mL, respectively. Terbinafine showed a growth inhibitory effect against *M. pachydermatis* at a concentration from 0.03 to 16 µg/mL.

Safety evaluation

The animals included in this study were physically examined

on each visit day, and owners were asked for any adverse reactions or side effects they had observed since the last visit. No adverse events were observed by the clinician or reported by the owners at any time point during the study.

Discussion

In this clinical study, the test solution was evaluated for safety and therapeutic efficacy *in vitro* and *in vivo*.

In vivo clinical efficacy of the otic solution was determined by TCS. On day 0, the TCS of the treatment and control group were 6.27 and 5.62, respectively, and no significant difference was observed between the 2 groups. After treatment, the TCS continuously decreased to 2.42 on day 14 and 1.85 on day 30 in the treatment group, while no relevant improvement of the TCS was observed in the control group (4.46 and 4.54 on day 14 and day 30, respectively). This result shows that the efficacy of the otic solution was maintained throughout 30 days from application.

The treatment success rate based on TCS (primary efficacy endpoint) was 88.5% in the treatment group with 23 out of 26 dogs successfully treated. On the contrary, only one out of 13 dogs was treated successfully in the control group, and overall success rate was 7.7%. TCS consisted of assessments of 4 clinical signs associated with inflammation. Mometasone furoate is a synthetic anti-inflammatory glucocorticoid [14]. The anti-inflammatory effect of mometasone furoate may reduce exuda-

Table 4. Proportion of clinical trial animals with reduced bacterial and fungal counts on day 30 of cytology

	Treatment group (n = 26)	Control group (n = 13)	Total (n = 39)
Reduced bacterial counts			
Success	23 (88.5)	3 (23.1)	26 (66.7)
Failure	3 (11.5)	10 (76.9)	13 (33.3)
Reduced fungal counts			
Success	25 (96.2)	3 (23.1)	28 (71.8)
Failure	1 (3.8)	10 (76.9)	11 (28.2)

Values are presented as number (%).

Table 5. MIC values of active agents against pathogens

Agent	Pathogen	Number	MIC range (µg/mL)	MIC ₅₀ (µg/mL)	MIC ₉₀ (µg/mL)
Florfenicol	<i>Staphylococcus pseudintermedius</i>	34	4–16	8	16
	<i>Pseudomonas aeruginosa</i>	11	2–16	16	> 16
	<i>Staphylococcus schleiferi</i>	7	2–8	8	8
	<i>Staphylococcus sciuri</i>	1	8	N	N
	<i>Corynebacterium auriscanis</i>	1	4	N	N
Terbinafine	<i>Malassezia pachydermatis</i>	50	0.03–16	0.06	0.125

MIC required to inhibit the growth of (MIC₅₀ = 50%; MIC₉₀ = 90%) the isolated.

MIC, minimum inhibitory concentration; N; no result.

tion and swelling and, as a result, increase the effectiveness of the antimicrobial agent. Therefore, this otic solution can effectively manage canine OE symptoms.

In cytologic evaluation, successful decrease in bacterial counts was observed in the treatment group. The reduction rate in the treatment group was considerably higher than in the control group (88.5% versus 23.1%). In terms of yeast count, the treatment group had a reduction rate of 96.2%, whereas the control had 23.1%. Therefore, we considered this otic solution effective in reducing the pathogen count, thereby improving the cytologic score.

The efficacy of the otic solution against pathogenic activity was also demonstrated *in vitro*. The MIC₅₀ values of florfenicol to suppress bacterial growth were 8 µg/mL, 16 µg/mL, and 8 µg/mL for *S. pseudintermedius*, *P. aeruginosa*, and *S. schleiferi*, respectively. The MIC₅₀ and MIC₉₀ of terbinafine to control *Malassezia* activity was 0.06 µg/mL and 0.125 µg/mL, respectively. Based on the MIC values obtained, the otic solution seems to be more effective on *Staphylococcus* spp. than *Pseudomonas* spp. In a previous study, the florfenicol concentration from an ear flush sample at 10 days after application of the otic solution was 8.7 µg/mL, whereas the terbinafine concentration at this time point was 4.8 µg/mL [15]. These concentrations were higher than the MIC₅₀ values of *Staphylococcus* spp. and *Malassezia* spp. in our study. Thus, we presume that one application of the otic solution can sustain efficacy over a few days.

S. pseudintermedius, *P. aeruginosa*, and *M. pachydermatis* are the most common causative pathogens in canine OE [3-7]. In the present study, *S. pseudintermedius* and *Pseudomonas* spp. were observed in 66.7% (26/39) and 17.9% (7/39) of the subjects, respectively. *Malassezia* was found in all cases. The most common multiple infections involved *S. pseudintermedius* and *M. pachydermatis*, which confirm the previous research findings. Thus, the combined efficacy of florfenicol and terbinafine in this solution may be considered as a first option for veterinarians to treat common dogs with OE.

Antimicrobial resistance affects the successful treatment of canine OE and can lead to refractory canine OE [3,19]. Our findings *in vivo* and *in vitro* demonstrate that this new otic solution is effective in managing canine OE, especially cases involving *S. pseudintermedius* and *Malassezia* spp. In addition, because this product is veterinarian administered, the chances of treatment failure by improper application or low compliance can be reduced. Finally, no safety related issues were reported during the study. Therefore, the combination of florfenicol, terbinafine, and mometasone furoate is highly recommendable in the treatment of canine OE.

Acknowledgments

This work was supported by Elanco Animal Health Korea Co., Ltd.

ORCID

Seulgi Bae, <https://orcid.org/0000-0001-9487-5665>

Yunseok Jin, <https://orcid.org/0000-0003-2246-6672>

Taeho Oh, <https://orcid.org/0000-0003-2898-6476>

References

1. O'Neill DG, Church DB, McGreevy PD, Thomson PC, Brodbelt DC. Prevalence of disorders recorded in dogs attending primary-care veterinary practices in England. *PLoS One* 2014; 9:e90501.
2. Hill PB, Lo A, Eden CA, Huntley S, Morey V, Ramsey S, Richardson C, Smith DJ, Sutton C, Taylor MD, Thorpe E, Tidmarsh R, Williams V. Survey of the prevalence, diagnosis and treatment of dermatological conditions in small animals in general practice. *Vet Rec* 2006;158:533-539.
3. Nuttall T. Successful management of otitis externa. In *Pract* 2016;38:17-21.
4. Miller WH, Griffin CE, Campbell KL. Disease of Eyelids, Claws, anal Sacs, and Ears. In: Muller and Kirk's Small Animal Dermatology. 7th ed. pp. 724-773, Elsevier, St. Louis, 2013.
5. Cole LK, Kwochka KW, Kowalski JJ, Hillier A. Microbial flora and antimicrobial susceptibility patterns of isolated pathogens from the horizontal ear canal and middle ear in dogs with otitis media. *J Am Vet Med Assoc* 1998;212:534-538.
6. De Martino L, Nocera FP, Mallardo K, Nizza S, Masturzo E, Fiorito F, Iovane G, Catalanotti P. An update on microbiological causes of canine otitis externa in Campania Region, Italy. *Asian Pac J Trop Biomed* 2016;6:384-389.
7. Hariharan H, Coles M, Poole D, Lund L, Page R. Update on antimicrobial susceptibilities of bacterial isolates from canine and feline otitis externa. *Can Vet J* 2006;47:253-255.
8. Cannon M, Harford S, Davies J. A comparative study on the inhibitory actions of chloramphenicol, thiamphenicol and some fluorinated derivatives. *J Antimicrob Chemother* 1990; 26:307-317.
9. Ganiere JP, Medaille C, Mangion C. Antimicrobial drug susceptibility of *Staphylococcus intermedius* clinical isolates from canine pyoderma. *J Vet Med B Infect Dis Vet Public Health* 2005;52:25-31.
10. Shin SJ, Kang SG, Nabin R, Kang ML, Yoo HS. Evaluation of

- the antimicrobial activity of florfenicol against bacteria isolated from bovine and porcine respiratory disease. *Vet Microbiol* 2005;106:73–77.
11. Guillot J, Bensignor E, Jankowski F, Seewald W, Chermette R, Steffan J. Comparative efficacies of oral ketoconazole and terbinafine for reducing *Malassezia* population sizes on the skin of Basset Hounds. *Vet Dermatol* 2003;14:153–157.
 12. Hofbauer B, Leitner I, Ryder NS. In vitro susceptibility of *Microsporum canis* and other dermatophyte isolates from veterinary infections during therapy with terbinafine or griseofulvin. *Med Mycol* 2002;40:179–183.
 13. Rosales MS, Marsella R, Kunkle G, Harris BL, Nicklin CF, Lopez J. Comparison of the clinical efficacy of oral terbinafine and ketoconazole combined with cephalexin in the treatment of *Malassezia* dermatitis in dogs: a pilot study. *Vet Dermatol* 2005;16:171–176.
 14. Reeder CJ, Griffin CE, Polissar NL, Neradilek B, Armstrong RD. Comparative adrenocortical suppression in dogs with otitis externa following topical otic administration of four different glucocorticoid-containing medications. *Vet Ther* 2008;9:111–121.
 15. Blake J, Keil D, Kwochka K, Palma K, Schofield J. Evaluation of a single-administration ototopical treatment for canine otitis externa: a randomised trial. *Vet Rec Open* 2017;4:e000219.
 16. Nuttall T, Bensignor E. A pilot study to develop an objective clinical score for canine otitis externa. *Vet Dermatol* 2014;25:530–e92.
 17. Weinstein MP. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. 11th ed. Clinical and Laboratory Standards Institute, Wayne, 2018.
 18. Alexander BD. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts. 4th ed. Clinical and Laboratory Standards Institute, Wayne, 2017.
 19. Paterson S. The use of antibiotics and antimycotics in otitis. *Companion Anim* 2018;23:608–613.