

Effect of Aromatherapy and Apupuncture on *Malassezia*-related Otitis Externa in Dogs

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Abstract : Aromatherapy and apupuncture was evaluated for its efficacy on canine *Malassezia*-related otitis externa. Ten dogs with *Malassezia*-related otitis externa were used in the present study. The control group was treated with ketoconazole, and the experimental group was treated with aromatherapy (topical aroma-oil drop) and apupuncture (injection-acupuncture of apitoxin). Clinical scores of the control group after 2-week treatment ($p < 0.01$) revealed a significant decrease compared with scores of pre-treatment. Experimental group scores after 2-week treatment revealed a significant decrease compared with scores of pre-treatment ($p < 0.05$). In the control group, ALT levels were significantly increased in the 1-week treatment ($p < 0.05$) and the 2-week treatment ($p < 0.01$), compared with the pre-treatment levels. The experimental group ALT levels did not significantly change after 1-week and the 2-week treatments, compared with pre-treatment. ALT levels of the experimental group after the 1-week treatment ($p < 0.05$) and the 2-week treatment ($p < 0.01$), was significantly lower than those of the control group. In conclusion, a combination therapy of apupuncture and topical aroma-oil drop is not hepatotoxic and similar to the effect of ketoconazole treatment for *Malassezia*-related otitis externa in dogs.

Key words : apupuncture, aromatherapy, *Malassezia* spp., otitis externa, dog.

Introduction

Malassezia is broad-based, budding, Gram-positive, lipophilic yeast that is frequently a normal inhabitant of the canine and feline external ear canal (6,10,14,19). In the canine ear canal, attachment to the cornified epithelial cell of *Malassezia* is mediated by lipids (17). *M. spp.* prefers the auditory canal of dogs with lipid-rich earwax where it grows rapidly (16). Although *Malassezia* organisms are typically peanut-shaped, they may be globose or ellipsoidal. Mean *Malassezia* counts per high-power dry field of ≥ 5 in the dog and ≥ 12 in the cat are considered pathologic. Mean *Malassezia* counts per high-power dry field of ≤ 2 in dog and cat are considered normal (18). *Malassezia* infections tend to produce copious, dark brown exudates with a sweet odor. *Malassezia* infections are a common cause of chronic otitis externa and treatment failure of otitis externa. Marshal *et al.* (15) reported that *Malassezia* spp. (82.8%) were isolated from 116 otitic dogs.

Aroma oils have the various medical properties. Bergamot oil and tea tree oil especially have a strong antifungal activity (1,23), and apitoxin has strong anti-inflammatory, antibacterial and antifungal effects (12). The present study was per-

formed in order to elucidate the therapeutic effect of a combination of aromatherapy and apupuncture on *Malassezia*-related otitis externa in dogs.

Materials and Methods

Animals

Client-owned ten dogs (six males and four females; age range: 3 to 13 years; weight range: 2.4 to 8 kg) with *Malassezia*-related otitis externa were used in the present study. The dogs were divided into the control group (ketoconazole treatment: 5dogs) and experimental groups (aromatherapy and apupuncture treatment: 5dogs). All dogs were treated with antihelminths and annual vaccination, and reared in the indoor. No underlying disease such as atopic dermatitis and food allergy was observed. No treatment received about otitis externa and other diaseses. Severity of otitis externa were severe in the control and experimental groups.

Diagnosis

Smears of external ear canal for cytologic evaluation were collected with sterilized cotton-tipped swabs. Diff-Quik stain was used for cytologic evaluation. *Malassezia* spp. was detected by microscopic examination. To confirm the *Malassezia* infections, the samples were inoculated on Sabouraud dextrose agar (SD agar[®], Asan pharmaceutical, Korea) at 30°C with an incubation period of 2-5 days and were identified by

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standard microbiological procedure. Each dog was re-evaluated at pre, 1 week and 2 weeks after treatment.

Treatment

The control group was medicated with ketoconazole (Kas-zol®, Cellat Pharm Korea; 10 mg/kg, PO, q12h) as an antifungal agent. Experimental group was treated with aroma-oil and apitoxin. Aroma-oil was blended following formula; 10 ml base oil (sweet almond; Aromatherapy Products Ltd., UK), 0.3 ml bergamot (*Citrus bergamia*; Aromatherapy Products Ltd., UK) and 0.2 ml tea tree (*Melaleuca alternifolia*; Aromatherapy Products Ltd., UK). Blended aroma-oil was applied 0.1 ml to ear canal in twice daily for 2 weeks. The dogs were injected with apitoxin solution in SI-19 (Ting Gong acupoint) bilaterally. An apitoxin solution was made from 1 mg apitoxin (Apimelena®, Samyang Anipharm Co., Korea). Apitoxin was diluted in 1 ml of normal saline and 0.1 ml of this solution was mixed with 0.1 ml of 2% lidocaine hydrochloride (Lidocaine HCl 2% Inj®, Daihan Pharm Co., Korea), and was diluted with normal saline (0.8 ml). The apitoxin solution was injected 0.2 ml into SI-19 (Ting Gong acupoint) with 24 hour intervals two days. All dogs' ear canals were cleaned using normal saline once in two days.

Clinical scores

Pruritus, cerumen, redness, odor and number of yeasts were chosen as the score calculation elements, and each element was divided into several phases (Table 1). Pruritus, cerumen, redness and odor in clinical score elements were evaluated by owners for objective estimation. Number of *Malassezia* was evaluated organism in high power field (× 400 magnification). Adding these elements, a score of 15 is perfect. Changes of clinical scores during the present study were recorded in pre-treatment, 1 week and 2 weeks after treatment.

Blood analysis

Blood samples were collected from cephalic vein by venipuncture. Serum levels of alanine transperase (ALT), alkaline phosphatase (ALKP), blood urea nitrogen (BUN), creatinine and total protein (TP) were determined using blood analyzer (VetTest 8008, IDEXX, USA). The blood analysis was determined before treatment, as well as 1 and 2 weeks after treatment, respectively.

Table 1. Clinical scores of the judged efficacy of a combination of aromatherapy and apipuncture

Score	Score calculation elements				No. of <i>Malassezia</i>
	Pruritus	Cerumen	Redness	Odor	
0	No	Little	No	No	No
1	Mild	Slight	Mild	Slight	1-4
2	Moderate	Moderate	Moderate	Moderate	5-9
3	Severe	Severe	Severe	Severe	> 10

Statistical analysis

Statistical significance was estimated using Mann-Whitney U of SPSS 12.0 K for Windows. A P value of < 0.05 was considered significant. Statistical significance compared to pre-treatment group in the clinical scores and ALT levels, and also statistical significance compared between control and experimental groups in the ALT levels.

Results

Clinical score and signs

Clinical scoring on each inspection day are shown in Table 1. Clinical scores in the control and experimental groups after treatment are shown in Fig 1. In the clinical scores of the control group, the 2-week treatment revealed a significant decrease in yeast infection compared with scores of pre-treatment ($p < 0.01$). Likewise in the experimental group, the 2-week treatment revealed a significant decrease compared with scores of pre-treatment ($p < 0.05$). There was no significant difference between control and experimental groups.

Blood analysis

Serum levels of ALT, ALKP, BUN, creatinine and TP were determined using a blood analyzer. In the control group, ALT levels increased significantly at the 1-week ($p < 0.05$) and the

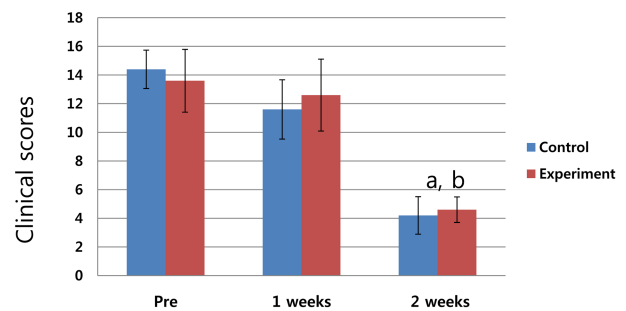


Fig 1. Clinical scores in the control and experimental groups (mean ± SD), Statistical significance compared to pre-treatment group (^a $p < 0.01$, ^b $p < 0.05$).

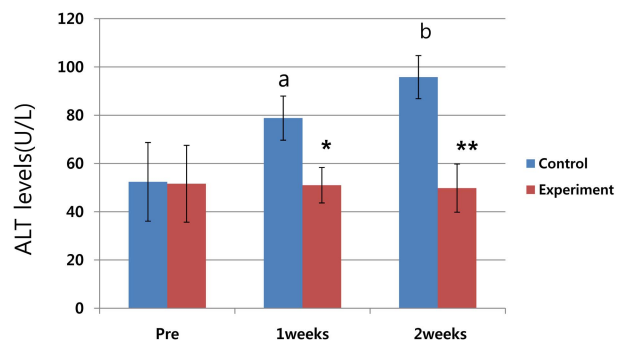


Fig 2. ALT levels in the control and experimental groups (mean ± SD), Statistical significance compared to pre-treatment group (^a $p < 0.05$, ^b $p < 0.01$), Statistical significance compared to control group (* $p < 0.05$, ** $p < 0.01$).

2-week ($p < 0.01$) exams, compared with those of pre-treatment group. In the experimental group, ALT levels were not significantly changed at the 1-week and the 2-week exams, compared with those of pre-treatment group. Comparing the two groups at the treatment exams, the experimental group's ALT levels were significantly lower than those of the control group at the 1-week ($p < 0.05$) and the 2-week exams ($p < 0.01$), (Fig 2). There were no significant differences in levels of ALKP, BUN, creatinine and TP between the control and experimental groups.

Discussion

Carfanchia *et al.* (2) reported that *Malassezia* yeasts were isolated from 72.9% of the cats and from 57.3% of the dogs with otitis externa. *Malassezia* spp. was frequently isolated from animals under 5 years of age. Chon *et al.* (3) reported that the higher prevalence of *Malassezia* spp. infection was observed in pendulous ear dogs (49.1%) as compared to that in the dogs with erect ears (23.6%).

Girão *et al.* (9) reported that 63.7% of the otitis specimens showed typical *Malassezia* cells on cytological examination. In samples taken from the healthy ears of dogs with unilateral otitis, only 21.43% showed evidence of *Malassezia*. *Malassezia* spp. was identified cytologically and culturally in 57.53%, 14.29% and 30.0% of samples from the ears of dogs with otitis, from the healthy ears of dogs with unilateral otitis and from the ears of healthy dogs with no otitis. In the present study, Diff-Quik stain was used for cytologic evaluation, and *Malassezia* spp. was detected by microscopic examination. We confirmed by all microscopic positive samples on Sabouraud dextrose agar. The poodle was the most common breed (39.29%) in the group with otitis associated with *Malassezia* spp. Like above findings, 8/10 dogs used in the present study have pendulous ear. The breeds were 3 Maltese, 4 Shih-Tzu and 1 Cocker Spaniel.

Some studies warn the possible zoonotic transmission of *Malassezia* to human beings from infected dogs (4,11,13,14, 20). In a human neonatal intensive care unit, *Malassezia* spp. was isolated from one or more clinical specimens. *Malassezia* spp. was cultured from blood, central venous catheter tips, urine, cerebrospinal fluid, eye discharge, ear discharge and tracheal aspirate (13). There is a report of chronic inflammatory skin granuloma caused by a *Malassezia*-infected dog (7). Therefore, it is important to diagnose correctly and treat effectively a canine malasseziosis for public health.

A number of researchers have examined treatment of *Malassezia*-related otitis externa in dogs. Morgan (18) reported that ketoconazole is effective for *Malassezia*-related otitis externa in dogs. Cole *et al.* (5) reported that neither ER (EDTA, tromethamine, benzyl alcohol) nor H₂O (purified water) had any effect on the growth of *Malassezia*. ER + keto (EDTA, tromethamine, benzyl alcohol, ketoconazole) was significantly more effective in reducing *Malassezia* growth at all-time points compared to both ER and H₂O. Also, Nakano *et*

al. (21) injected 0.5 ml of beta-thujaplicin solution of 100 µg/ml including DMSO 2% daily into both external ear canals of 31 cases. The beta-thujaplicin eardrops were effective for *Malassezia*-related otitis externa in dogs.

In this study, the control group was medicated with ketoconazole as an antifungal agent. By the clinical scores of control group, the 2-week treatment revealed a significant decrease compared with scores of pre-treatment, but, ALT levels significantly increased after 1-week and 2-week treatment in this group, because ketoconazole has hepatic toxicity (22). In the experimental group, the authors used apipuncture and aromatherapy that have been known to have antifungal effects. The clinical scores of the apipuncture and aromatherapy group, revealed a significant decrease at 1 and 2 weeks compared with scores of pre-treatment. ALT levels remained within normal range after treatment in this group. The results showed that apipuncture and aromatherapy have no hepatotoxicity in the present study. ALT levels in the experimental group were significantly lower during the treatment period than in the control group. Moreover, two dogs of control group have persistent ALT elevation over the normal range after the 2-week treatment. The results suggest that a combination therapy of apipuncture and topical aroma-oil drop is not hepatotoxic, and similar to the effect of ketoconazole treatment for *Malassezia*-related otitis externa in dogs. The limitation of this study was not a lot of samples for *Malassezia*-related otitis externa in dogs.

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개 말라세지아성 외이염에 대한 아로마치료와 약침의 치료 효과

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요 약 : 말라세지아성 외이염에 대한 아로마치료법 (아로마오일)과 약침치료법 (아피톡신)을 적용하여 임상증상 개선 효과를 알아보기 위해 본 연구를 실시하였다. 실험군 5두는 아로마치료와 약침을 실시하였고 대조군 5두는 케토코나졸을 적용하였다. 임상증상은 대조군과 실험군에서 치료 전에 비해 각각 치료 후 2주 후에 유의성 있는 개선효과를 나타내었다 ($p < 0.01$, $p < 0.05$). ALT 수치는 대조군에서 치료 전에 비해 치료 후 1주, 2주 후에 각각 유의성 있는 증가 소견을 나타내었다 ($p < 0.05$, $p < 0.01$). 실험군의 ALT 수치의 변화는 대조군에 비해 치료 1주 후, 2주 후에 각각 유의성 있는 감소소견을 나타내었다 ($p < 0.05$, $p < 0.01$). 결론적으로 말라세지아성 외이염에 대한 아로마치료법(아로마오일)과 약침치료법은 효과가 있으며 대조군에 비해 간독성은 적은 것으로 나타났다.

주요어 : 약침, 아로마치료, 말라세지아, 외이염, 개