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Efficacy of apitoxin for the treatment of otitis externa in dogs

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Abstract : This study examined the efficacy of apitoxin for the treatment of otitis externa in dogs. Ten dogs with otitis externa were allocated randomly to two groups. The control group was treated with the susceptible antibiotics and the experimental group was injected with apitoxin into the tragus subcutaneously. There were no significant differences in the clinical scores, blood WBC counts and neutrophil/lymphocyte ratios between the control and experimental groups. By 2 weeks, the bacterial cell counts were significantly lower in the experimental group than the control ($p < 0.05$). No adverse reactions were observed in any of the dogs during the study. This suggests that a topical injection of apitoxin is an effective treatment for otitis externa in dogs.

Keywords : apitoxin, canine, non-acupoint, otitis externa

Introduction

Canine otitis externa is a common in small animal clinical practice and can be a problem to manage. In particular, infections with bacteria and yeast are perpetuating factors that prevent the ear canal from effective healing. Bacteria and yeast colonize and reproduce due to changes in the ear canal that occur as a result of disease [8, 17, 24, 25].

Bacteria and yeast are the major factors of otitis externa in dogs. Some studies reported the microbial flora isolated from otitic dogs. *Malassezia* spp. (54.2%), coagulase-positive *Staphylococcus* spp. (32%), *Proteus* spp. (9%) and *Pseudomonas* spp. (9%) were isolated from 115 otitic dogs [26]. Also, *Malassezia* spp. (82.8%), coagulase-positive *Staphylococcus* spp. (37.9%), *Pseudomonas* spp. (16.4%), *Streptococcus* spp. (8.6%) and *Proteus* spp. (3.4%) were isolated from 116 otitic dogs [18].

Bee-venom (apitoxin) has strong anti-bacterial, anti-fungal and radioprotective effects that are consistent with its strong anti-inflammatory effects [12, 14, 21, 27]. Peptides, such as mellitin, apamin, peptide 401, adolapin and protease inhibitors, are the main pharmacological components that reduce inflammation. In addition to its anti-inflammatory properties, apitoxin is a strong immunologic agent that stimulates the protective mechanisms of the body against disease [10, 14-16]. Its main component, mellitin, also has anti-inflammatory and antibacterial effects. Mellitin stimulates the hypophyseal-adrenal system and releases 100 times more cortisone than hydrocortisone [1, 14, 27, 28]. Matsuzaki reported that the antibacterial activity of bee venom was due to the permeability of the bacterial membranes. Melittin also translocates across the membrane by forming a pore. The membrane selectivity of these peptides is closely related to their affinity for the lipids constituting the membrane surface [19]. Bee venom produces a sustained increase in serum corticosterone concentrations, and disease suppression may be mediated through the pituitary-adrenal axis [30].

The therapeutic effects of apitoxin in veterinary medicine have been investigated using injection-acupuncture in horse, pigs, calves and canine hind limb paralysis [2-5, 11, 13].

In this study, the therapeutic effects apitoxin on otitis externa in dogs was evaluated using non-acupoints.

Materials and Methods

Animals

Ten dogs (eight males and two females; age range:

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1 to 3 years; weight range: 3.1-4.5 kg) that presented with otitis externa were used in this study. The dogs were divided randomly into a positive control group (antibiotics treatment: five dogs) and experimental group (apitoxin treatment: five dogs).

Treatment

The positive control group was medicated with amoxicillin-clavulanic acid 12.5 mg/kg (Clabulo duo syr; Il yang Pharm, Korea) and ketoconazole 10 mg/kg (Kaszol; Cellat Pharm Korea, Korea) according to the results of an antibiotics sensitivity test and bacterial isolation. The experimental group was injected with an apitoxin solution into tragus subcutaneously. An apitoxin solution was made from 1 mg apitoxin (Apitoxin; Guju Pharmacological, Korea). Apitoxin was diluted in 1 ml of normal saline. 0.1 ml of this solution was mixed with 0.1 ml of 2% lidocaine hydrochloride (Lidocaine HCl 2% Inj; Daihan Pharm, Korea), and diluted with normal saline (0.8 ml). The apitoxin solution (0.2 ml) was injected into the tragus subcutaneously once every two days regardless of body weight using 1 ml syringes with a 26G needle. The ear canal of all dogs were cleaned using normal saline once every two days.

Bacterial isolation

Samples were collected from the ear canal of all dogs prior to treatment. All bacterial strains obtained were isolated and identified using the standard method and VITEC identification system (BioMeriux, France).

Total bacterial cell count

The bacteria isolated from the sample were suspended in 1 ml PBS. One hundred μl was then spread over brain heart infusion agar (BHI agar) and incubated at 37°C for 48 h. The total bacterial colony forming units (CFU) were then counted before treatment, as well as at 1 and 2 weeks after treatment.

Clinical scores

Pruritus, cerumen, redness, swelling, heat, pain and odor were used as score calculation elements. Each element was divided into several phases (0; no or little, 1; mild or slight, 2; moderate, 3; severe). These element phases were added and a score of 21 was considered a perfect score. The changes in the clinical scores during this study were recorded before treatment, as well as at 1 and 2 weeks after treatment.

Blood analysis

Blood samples were collected from the cephalic vein by venipuncture. The total WBC count and neutrophil/lymphocyte (N/L) ratio were determined using an automatic cell counter (MS9-5V; Melet Schloesing Laboratories, France). Blood analysis was performed before treatment, as well as at 1 and 2 weeks after treatment.

Statistical analysis

In this study, the statistical significances between the groups were analyzed using a Mann-Whitney U of SPSS 12.0 K for Windows ($p < 0.05$).

Results

Bacterial isolation

The distribution of bacterial isolates from the otitic lesions was *Staphylococcus* spp. and *Streptococcus* spp. (3/10), *Staphylococcus* spp. and *Pseudomonas* spp. (3/10), *Staphylococcus* spp. only (2/10) and *Staphylococcus* spp. and *Malassezia* spp. (2/10).

The change of bacterial cell count

The positive control group showed a slightly lower bacterial replication rate after treatment with the susceptible antibiotics (before: 6.2 ± 0.8, 1 week: 3.3 ± 0.1 and 2 weeks: 1.4 ± 0.5 log CFU/ml). The experimental group showed a considerably lower bacterial replication rate (before: 5.9 ± 0.7, 1 week: 2.9 ± 0.5 and 2 weeks: 0.7 ± 0.5 log CFU/ml). Two weeks after treatment, the bacterial cell counts in the experimental group were significantly lower than the positive control ($p < 0.05$) (Table 1).

Clinical score

The clinical signs including pruritus, cerumen,

Table 1. The change of bacterial cell counts

Group	Days after treatment		
	Pre	1 week	2 weeks
Positive control	6.2 ± 0.8	3.3 ± 0.1	1.4 ± 0.5
Experimental	5.9 ± 0.7	2.9 ± 0.5	0.7 ± 0.5*

Results are shown as the mean ± SD. *Significant difference between positive control and experimental group ($p < 0.05$).

Table 2. The change of clinical signs

Group	Days after treatment		
	Pre	1 week	2 weeks
Positive control	19.6 ± 2.1	2.6 ± 0.6	0.8 ± 0.5
Experimental	18.8 ± 0.5	3.2 ± 1.9	0.4 ± 0.6

Results are shown as the mean ± SD.

Table 3. The change of total WBC counts

Group	Days after treatment		
	Pre	1 week	2 weeks
Positive control	13.3 ± 3.2	13.4 ± 2.7	12.6 ± 2.2
Experimental	15.5 ± 2.8	15.5 ± 1.5	13.8 ± 2.3

Results are shown as the mean ± SD.

Table 4. The change of neutrophil/lymphocyte ratios

Group	Days after treatment		
	Pre	1 week	2 weeks
Positive control	2.9 ± 1.2	3.9 ± 1.4	3.0 ± 0.7
Experimental	2.6 ± 1.3	2.7 ± 1.1	1.8 ± 0.9

Results are shown as the mean ± SD.

redness, swelling, heat, pain and odor were greatly improved after the 2-week treatment in the experimental and positive control groups. There were no significant differences in the clinical score between the positive control and experimental groups (Table 2).

Total WBC counts and N/L ratios

The total WBC counts and N/L ratios were measured using a blood cell counter analyzer. There were no significant differences in the changes in the blood WBC counts (Table 3) and N/L ratios (Table 4) between the positive control and experimental group.

Discussion

In this study, 0.2 ml of the apitoxin solution was injected into the tragus subcutaneously once every two days over a 2-week period. A subcutaneous injection of apitoxin into the tragus was effective in treating otitis externa. The clinical signs improved significantly after the 2-week treatment in the apitoxin and antibiotics treatment groups. The bacterial cell count in the apitoxin group decreased significantly after the 2-week treatment.

Many attempts have been made to prevent antibiotics resistance in otitis externa in dogs. New approaches were carried out on effective topical therapies for otitis externa. A topical application of bacitracin or chloramphenicol [9], beta-thujaplicin ear drops for *Malassezia*-related canine otitis externa [20], the new pH-balanced propylene glycol-free test ear cleanser [22], an ear rinse containing tromethamine, EDTA, benzyl alcohol and 0.1% ketoconazole [7] and the ear cleanser containing 2.5% lactic acid and 0.1% salicylic acid [6] have been used to help solve the problem of antibiotic resistance as well as to increase the therapeutic success for the treatment of otitis externa in dogs.

In this study, the use of apitoxin in injection-acupuncture was evaluated as a topical treatment for otitis externa in dogs. Commercial apitoxin was developed containing mainly mellitin, which is administered as an intradermal injection to acupoints. This study examined its efficacy using non-acupoints, the tragus subcutaneously.

Zerbini *et al.* [29] reported that mellitin has good *in vitro* activity against Gram-positive and Gram-negative bacteria, and is active against *Mycobacterium smegmatis in vitro*. However, they did not report any activity against the other mycobacteria analyzed. In addition, cecropin A-melittin peptides were reported to be a promising alternative to overcome the polymyxin resistance in *Acinetobacter baumannii* [23].

There has been increasing interest in complementary and alternative medicine due to the misuse or abuse of antibiotics and an increase in the number of antibiotics-resistant bacteria. Resistance to antibiotics is an important issue in public health and animal welfare.

In this study, non-acupoints, tragus, were chosen for the treatment of otitis externa in order to make this treatment available to those with little experience in acupuncture. The clinical signs of the dogs improved favorably after the subcutaneous injection of apitoxin. No adverse reactions, such as anaphylactic shock, were encountered during the study. In contrast to concerns, there was less or similar injection pain to an intramuscular or subcutaneous injection. The clinical signs, including pruritus, cerumen, redness, swelling, heat, pain and odor, were greatly improved after the 2-week treatment in both the experimental and positive control groups. There were no significant differences in the changes in the blood WBC counts and N/L ratios between the positive control and experimental groups. After the 2-

week treatment, the bacterial cell counts in the apitoxin group was significantly lower than in the antibiotics group ($p < 0.05$).

The small number of dogs used was a limitation to this study. Therefore, further studies will be needed to support these results and evaluate the safety of apitoxin.

In conclusion, apitoxin is an effective treatment for otitis externa in dogs. In addition, an apitoxin therapy may be an alternative treatment for otitis externa in dogs.

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