A THESIS FOR THE DEGREE OF MASTER

In vitro antifungal activity of cold atmospheric microwave plasma against *Malassezia pachydermatis*

*Malassezia pachydermatis*에 대한 저온 마이크로파 플라즈마의 항진균 작용

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이 태 현

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In vitro antifungal activity of cold atmospheric microwave plasma against *Malassezia pachydermatis*

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Abstract

Malassezia pachydermatis is a commensal yeast that can cause skin disease like *Malassezia* dermatitis in veterinary medicine. Cold atmospheric microwave plasma (CAMP) has been used in disinfection in medicine over the past few years. Although *M. pachydermatis* is one of the most common isolated fungi for skin disease and otitis externa

in dogs, there is no data available regarding the antifungal effect of cold atmospheric microwave plasma against *M. pachydermatis.* The objective of this study was to evaluate the antifungal efficacy of CAMP and the synergistic effect against *M. pachydermatis* combined with chlorhexidine gluconate. A *M. pachydermatis* isolate was collected from a canine patient at the Veterinary Medical Teaching Hospital of Seoul National University. Antifungal effect was determined by application of CAMP for a *M. pachydermatis* isolate that was incubated for 3 days at 37℃. After 1, 2, 3 and 5 minutes of application, efficacy of plasma treatment was determined by the number of colony forming units (CFU). A mixture consisting of inoculum and chlorhexidine gluconate was applied for evaluating synergistic effects with plasma treatment in the same way. The application of CAMP showed great antifungal effects against *M. pachydermatis*. The antifungal effect of CAMP was enhanced by an increased exposure time and output power. The application of CAMP with 0.02% and 0.2% CHX resulted in lower survival rates against *M. pachydermatis* when compared with its sole application at 1 or 2 minutes. The study demonstrated that CAMP may be a great new antifungal option for *M. pachydermatis.* Further clinical studies are required to assess *in vivo* efficacy for patients.

Key words: Canine, cold atmospheric microwave plasma, antifungal activity, *Malassezia pachydermatis*, Chlorhexidine gluconate

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1. Introduction

Malassezia pachydermatis is one of the most commonly isolated fungi that causes skin disease and otitis externa in dogs [1,2]. *M. pachydermatis* is a commensal yeast that commonly colonizes the superficial layers of the epidermis; it is the predominant organism in the skin [3]. *M. pachydermatis* is classified as a lipophilic, non-lipid dependent, nonmycelial saprophytic yeast that is commonly found on the skin, in ear canals, and on mucosal surfaces including the lips, chin, anal sacs in the rectum, and vagina. The most common clinical sign of *Malassezia* dermatitis is erythema, commonly with seborrhea (greasy material), hyperkeratinization and pruritus. Other possible signs are hyperpigmentation, lichenification, malodor, and otitis externa [4]. In dogs, atopic dermatitis is usually associated with *Malassezia* dermatitis, and in atopic dogs, *Malassezia* overgrowth can exacerbate hypersensitivity [5]. *M. pachydermatis* has a thick (up to 0.25 um) and multilamellar cell wall, similar to other *Malassezia* yeasts [3]. Although the components of *M. pachydermatis* have been poorly studied, polysaccharides were found to contribute to the rigidity of the cell wall in *Malassezia spp.* in a previous study [6]. Because of the crossed electron-translucent bands with helicoidal ridges, *M. pachydermatis* withstands cell changes, osmotic pressure, and other environmental changes [6].

Plasmas represent the fourth state of matter (the first three being solid, liquid, and gas) and is generated through the ionized gas by electric fields [7-9]. In general, heat is generated by plasma at high temperatures. However, cold atmospheric microwave plasma (CAMP) generates heat below 40°C; thus, devices using this plasma have been applied in medicine [9]. One of the main purposes of these applications is to kill parasites, bacteria, fungi, and viruses; other purposes include blood coagulation, sterilization, and wound healing [10-12]. Several studies have assessed the bactericidal and fungicidal efficacies of various types of plasma [10-12]. One reasonable hypothesis states that plasma can destruct the membrane that derives its tensile strength from electrostatic forces that increase as charge accumulates [7,13]. CAMP also decreased bacterial and fungal growth in a previous study [10]. Therefore, CAMP has potential as a new bactericidal and fungicidal agent. However, although *M. pachydermatis* is a commensal fungi for all dogs regardless of the presence of skin problems [14], no *in vitro* studies have yet been conducted related to any plasma treatment against *M. pachydermatis*, unlike studies on the soil-borne fungal pathogen *Fusarium oxysporum* and the normal human fungus *Candida albicans* [15,16]. Chlorhexidine gluconate (CHX), a bisbiguanide antiseptic, has shown antimicrobial effect against many fungi and enveloped viruses [17]. In combination with other antifungals such as miconazole in shampoo, CHX has also shown remarkable antifungal effects [18,19].

The purpose of this study was to evaluate the antifungal potential of CAMP against *M. pachydermatis* and to confirm its synergistic effect with CHX, which has already been confirmed to have antifungal efficacy.

2. Materials and Methods

2.1. Malssezia pachydermatis isolate

An *M. pachydermatis* isolate was collected from a canine patient with otitis externa and Malassezia dermatitis at the Veterinary Medical Teaching Hospital of Seoul National University. Ear discharge was collected using a sterile cotton swab and transported by transport medium to the laboratory. The isolate had been identified as *M. pachydermatis* in a previous molecular study [20]. In that study, the isolate was identified as *M. pachydermatis* and underwent a sequence analysis of the internal transcribed spacer 1 (ITS-1) and the intergenic spacer 1(IGS-1) of rDNA [20]. The *M. pachydermatis* isolate was cultured on sabouraud dextrose agar (SDA; Sigma-Aldrich; Milan, Italy) and incubated for three days at 37°C [2,18,21].

2.2. In vitro antifungal susceptibility testing for cold atmospheric microwave plasma

Cold atmospheric microwave plasma (CAMP; IonMedical Inc.; Seongnam, Korea), which consists of a handpiece jet pen in which the plasma is generated by atmospheric pressure, output power (W), and a gas flow is used for this study (Figure 1). Argon gas was applied in this device, and the parameters were set to a

temperature below 40°C, 10-15 LPM (liters per minute), and 3–50 W with 2450 MHz, 3.5 kV.

Stock inoculum suspensions were prepared using a three-day-old colony cultured on SDA. The colony was suspended in 3 ml of sabouraud dextrose broth (SDB; Sigma-Aldrich; Milan, Italy) until reaching an optical density of 0.5 McFarland using a turbidimeter (Densichek McFarland Densitometer, Biomerieux, Lyon, France) (equivalent to 7–9 x 10^5 colony forming units (CFUs)/ml as validated by quantitative plate counts of CFUs in SDA) [1,21]. The inoculum was diluted 100-fold with SDB. Ten microliters of the inoculum was plated onto the SDA, and each agar was treated for 1, 2, 3, and 5 min with 10 or 15 LPM and 30 or 50 W, respectively. After plasma treatment, each plate was incubated for three days at 37°C, and the total number of CFUs was determined. Plasma application without ignition (argon gas only) was used as a negative control. At least three independent tests were conducted for the evaluation of CFU.

2.3. In vitro antifungal susceptibility testing for combination with cold atmospheric microwave plasma and chlorhexidine gluconate

A 5% aqueous solution of CHX (Greenpharmaceutical; Chungchungbuk-do, Korea) was used. The CHX was diluted to 0.02%, 0.2%, and 2% with distilled water. The *in vitro* antifungal activity methods were modified from a previous study

[18,21]. The inoculum (equivalent to 7–9 x 10^5 CFU/ml as validated by quantitative plate counts of CFUs in SDA) and each concentration of CHX was added to each conical tube (SPL; Gyeonggi-do, Korea) in equal quantities (1.5 mL). Each tube was left at room temperature for 1, 2, 3, and 5 min. After 1, 2, 3, and 5 min, the mixtures were transferred to SDB, and the mixtures were finally diluted so that the inoculum was diluted 100-fold with SDB. Ten microliters of the inoculum was plated onto SDA and incubated for three days at 37°C, after which the total number of CFUs was determined. All tests were performed at least triplicate for the evaluation of CFU.

The combination methods were modified from a previous study that had determined the CFUs by treating the plasma in broth medium followed by inoculation on an agar plate [22]. Similar to testing for antiseptics, each final diluted mixture that was added to the inoculum and each concentration of CHX (i.e., 0.02%, 0.2%, and 2%) was placed in the wells of a 24-well sterile cell culture plate (Falcon, Corning, USA). Each mixture in the wells was applied to the plasma for 1, 2, 3, and 5 min. After 1, 2, 3, and 5 min, 10 μL of the inoculum was plated onto SDA and incubated for three days at 37°C, after which the total number of CFUs was determined. CAMP was applied at 50 W with 15 LPM. The combination tests were conducted at least three times for the evaluation of CFU.

2.4. Statistical analysis

SPSS Statistics 23 (IBM Corporation, Armonk, NY, USA) was used to perform all statistical comparisons. To determine the antifungal effect of CAMP and to evaluate the synergistic effect between CAMP and CHX for each trial time, the Kruskal–Wallis test was used. If the antifungal effect and the synergistic effect were confirmed, the Mann–Whitney *U* test was used to compare each condition for each trial time. Statistical significance was defined as $p < 0.05$.

3. Results

3.1. Determination of the antifungal effect against M. pachydermatis for cold atmospheric microwave plasma

The CFU values are described in Table 1 as the survival rate that was compared with the negative control without plasma exposure at 100%. The survival rates were significantly reduced in all conditions of CAMP (30 W with 10 LPM, 30 W with 15 LPM, 50 W with 10 LPM, and 50 W with 15 LPM) for 3 and 5 minutes ($p < 0.05$). No significant difference was observed in the efficacy of CAMP for 1 or 2 minutes ($p > 0.05$).

For 3-minute CAMP application, all conditions except 30 W with 15 LPM demonstrated better antifungal effects than argon gas alone ($p < 0.05$). In the conditions in which CAMP was applied at 50 W with 15 LPM, the survival rate was 10.33±3.51%. It demonstrated significantly less survival rate than the other conditions of CAMP ($p < 0.05$) except the conditions in which CAMP was applied at 50 W with 10 LPM ($p > 0.05$). The CFU values in these two conditions (50 W with 15 LPM, and 50 W with 10 LPM) did not differ significantly ($p > 0.05$).

In all conditions in which CAMP was applied for 5 minutes, the survival rates were greatly reduced comparing with 1, 2, and 3 minutes. At 30W, the survival rates were 17±7.55% in 10 LPM, 13.67±5.77% in 15 LPM. Also, the survival

rates were 3.67±4.62% in 10LPM, 5±4.58% in 15 LPM. Then there was no significant difference with all conditions of CAMP ($p > 0.05$). And there were shown better antifungal effects than the condition in which plasma without ignition ($p < 0.05$).

3.2. Evaluation of the synergistic antifungal effect against Malassezia pachydermatis between cold atmospheric microwave plasma and chlorhexidine gluconate

The antifungal effects of the concentrations of CHX with or without plasma application are described in Table 2 as the survival rate that was compared with the negative control without plasma exposure at 100%.

With regard to exposure times, the survival rates were significantly reduced by CHX and CHX with CAMP for 1, 2, 3 and 5 minutes ($p < 0.05$). With increasing concentration of CHX (i.e., 0.02%, 0.2%, and 2%) for 1 and 2 minutes, the antifungal effects of CHX increased ($p < 0.05$).

Because no *M. pachydermatis* colony was observed either at 2% CHX or 2% CHX with CAMP, other concentrations of CHX (i.e., 0.02% and 0.2%), both alone and with CAMP, were evaluated for synergistic antifungal effects against *M. pachydermatis*. For 1 minute, when CAMP was combined with 0.02% CHX, the survival rate was 15.33±11.59% that was compared with 39.67±13.01% at the application of 0.02% CHX only. Also, when CAMP was combined with 0.2% CHX,

the survival rate was 1±1.73% that was compared with 5.67±3.79% at the application of 0.2% CHX only. The application of both 0.02% and 0.2% concentrations of CHX with CAMP produced synergistic antifungal effects, as evidenced by the reduction of survival rates ($p < 0.05$). The application of 0.02% CHX with CAMP reduced survival rates more significantly than the application of 0.02% CHX alone ($p < 0.05$). Except at a concentration of 0.02%, CHX alone reduced CFU values more significantly than CAMP alone ($p < 0.05$).

For 2 minutes, when CAMP was combined with 0.02% CHX, the survival rate was 19±6% that was compared with 28.67±5.03% at the application of 0.02% CHX only. The application of both 0.02% CHX and CAMP produced synergistic antifungal effects as evidenced by the reduction of CFU values ($p < 0.05$). Although CAMP combined with 0.2% CHX decreased more CFU than 0.2% CHX only, there was no significant difference between 0.2% CHX alone and 0.2% CHX with CAMP ($p > 0.05$).

For 3 and 5 minutes, no significant difference was observed in the synergistic antifungal effect between CHX and CAMP ($p > 0.05$). When CAMP was applied alone, the survival rate was 10.33±3.51% for 3 minutes and 5±4.58% for 5 minutes that were compared with 27.33±10.69% and 23.67±13.45% for same time at the application of 0.02% CHX only. The application of CAMP alone showed more antifungal efficacy than 0.02% CHX alone and less antifungal efficacy than both 2%

CHX alone and 2% CHX with CAMP ($p < 0.05$).

In addition, except application for 1 minute, the effects of 0.2% CHX alone differed significantly from those of 0.02% CHX with CAMP ($p < 0.05$). Except for application for 1, 2 minutes, CAMP alone reduced CFU values more than 0.02% CHX alone ($p < 0.05$).

4. Discussion

Cold atmospheric microwave plasma has recently been considered an important application for disinfection in medicine [23]. Previous studies indicated the bacterial inactivation mechanism by which plasma-emitted particles can destroy the cell envelope [9]. Compared to bacteria, fungi have a different cell wall structure, membrane, and complex defense mechanisms against various pressures [15]. *M. pachydermatis* is primarily responsible for fungal skin diseases and atopic dermatitis in veterinary practice. Although *M. pachydermatis* is a commensal fungi for all dogs regardless of skin problems [14], no *in vitro* studies have yet been conducted to evaluate plasma treatment against *M. pachydermatis* in veterinary medicine. Because *M. pachydermatis* has a thick and multilamellar cell wall [3], this study was worthwhile in evaluating the *in vitro* activity of plasma against *M. pachydermatis*.

This study identified that CAMP showed a significant antifungal effect against *M. pachydermatis* (Table 1). We confirmed that CAMP requires the ignition process for effective antifungal activity against *M. pachydermatis*. A previous study had indicated that there was a difference in antiseptic efficacy depending on the presence of ignition [22]. Similarly, this study showed that the ignition process should be included in plasma applications for consistent and effective antifungal activity.

This study confirmed that CAMP requires longer application times to achieve a greater antifungal efficacy against *M. pachydermatis.* In our study, CAMP applied for over 3 minutes produced statistically significant antifungal effects against *M. pachydermatis*. The lack of statistical significance for the 5-minute application suggests that the antifungal effect was efficacious for all conditions of CAMP at 5 min. In summary, clinical antifungal effects against *M. pachydermatis* are expected after over 3 minutes of plasma application. In this study, complete decontamination was not achieved within 5 min. Similar to a previous study on human pathogenic dermatophytes, there was significant decontamination of *Trichophyton rubrum, Trichophyton interdigitale, Arthroderma benhamiae*, and *Microsporum gypseum* after a few minutes of plasma application, but complete decontamination was not achieved in *M. gypseum* [24].

Compared with other studies on plasma application against bacteria regardless of plasma type, longer time was required so that antifungal effects are significantly shown after treating fungi with plasma, even when using a low output power [7,23,25]. These results imply that fungi have a complex structure including a very thick and rigid cell wall, which was reported in a previous study on *C. albicans* [25]. Difference in plasma devices and application methods can also influence these results. Furthermore, our study found that a stronger output power for CAMP can lead to more effective antifungal activity against *M. pachydermatis*.

This study also reported a synergistic antifungal effect of CAMP and CHX against *M. pachydermatis* (Table 2). Because CHX was reported to be effective against *M. pachydermatis in vitro* and *in vivo,* CHX is a good partner for evaluating plasma application as an antiseptic. When 2% and 0.2% CHX were used, the antiseptic effects against *M. pachydermatis* were always stronger than those of CAMP.

When evaluating survival rates after the sole application of CAMP or CHX, the longer CAMP is applied, the greater the difference in antiseptic effect compared with 0.02% CHX. Moreover, the longer CAMP is applied, the smaller the gap in antiseptic effect compared with 0.2% and 2% CHX. Therefore, CAMP showed a remarkable increase in antifungal effect activity over time. A previous study using 0.1% CHX also showed that antiseptic effects against *Streptococcus mutans* between 0.1% CHX and plasma treatment were similar or slightly lower with the plasma treatment [22]. This finding implies that if the plasma application time is much longer, the decontamination level can be similar to that achieved with 0.2% and 2% CHX.

The 1- and 2-minute applications with 0.02% CHX and CAMP produced better synergistic antifungal effects than those of 0.02% CHX alone. Also, the 1-minute application with 0.2% CHX and CAMP produced synergistic antifungal effects. Because *Malassezia* overgrowth is often accompanied by atopic dermatitis that necessitates continuous lifelong care, various combinations and options for topical

treatment are necessary. As CHX is a broad-spectrum antiseptic for microorganisms such as bacteria and fungi, CHX was used in this study to determine its synergistic effect with CAMP. Although a previous study compared the antibacterial activity of plasma with antiseptic agents including CHX, it didn't reveal any antifungal efficacy and synergistic activity [26]. Additionally, it is necessary to determine the synergistic effect of CAMP with a specific antifungal agent against *M. pachydermatis* such as miconazole in future studies.

There was a limitation in this study. Because of the small sample size, the 5-minute applications of with 0.02% CHX and CAMP produced less antifungal effects than those of CAMP. Therefore a large number of *M. pachydermatis* clinical isolates and standard strains of *M. pachydermatis* are required for further study. However, because there are no other plasma studies related to *M. pachydermatis* in veterinary medicine, this study has great significance in showing its antifungal efficacy against *M. pachydermatis*. Therefore, this study highlights a new potential antifungal agent. Further studies are necessary to investigate the physical mechanism of *M. pachydermatis* destruction and to assess its efficacy *in vivo*.

5. Conclusions

The present study has demonstrated that cold atmospheric microwave plasma makes antifungal effects *in vitro* against *M. pachydermatis.* And this study has also confirmed that cold atmospheric microwave plasma has synergistic antifungal effects *in vitro* against *M. pachydermatis* when combined with chlorhexidine gluconate. Clinical studies will be required to assess the *in vivo* efficacy.

Figure 1. Cold atmospheric microwave plasma (CAMP) device

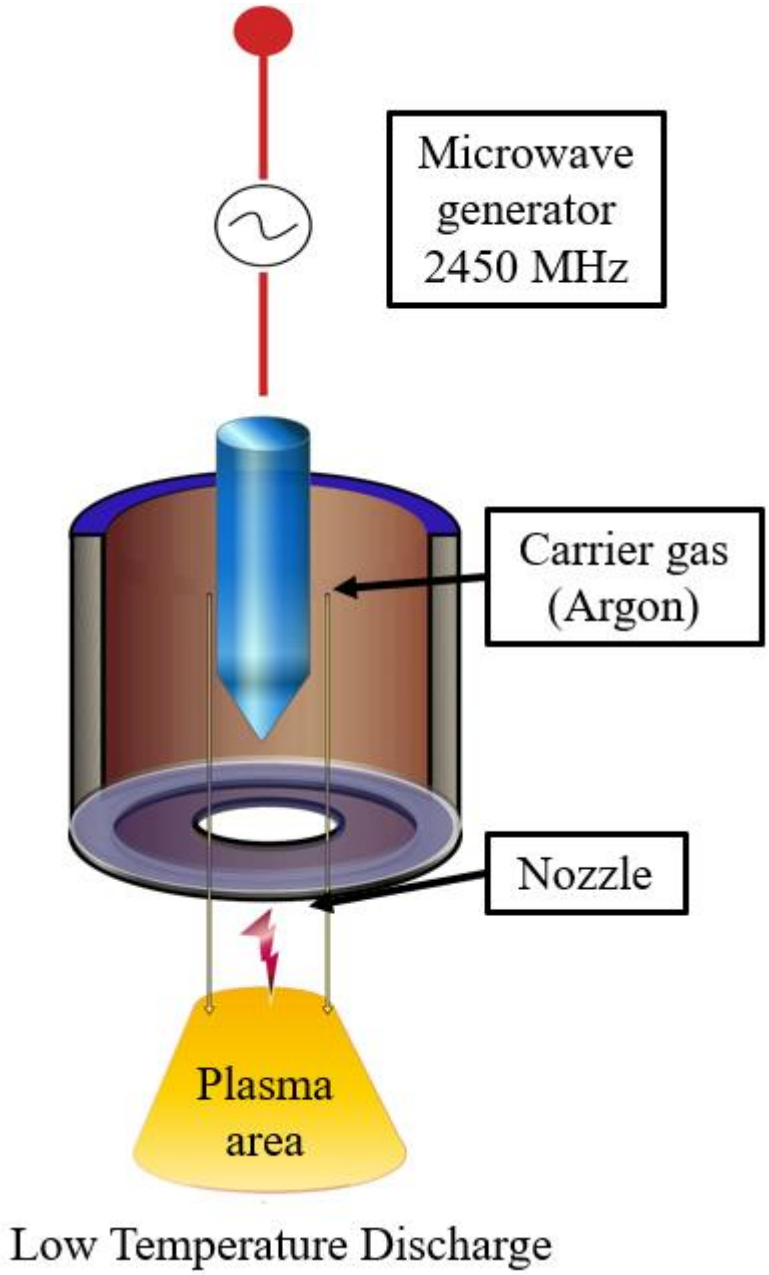

Table 1. Survival rates of *Malassezia pachydermatis* colony forming units (CFU) after cold atmospheric microwave plasma

	LPM_W				
time	10LPM_30W	10LPM_50W	15LPM_30W	15LPM_50W	Ar gas only
1 minute	63±16.64	50.33±28.01	76.33±12.59	36±10	49.66±19.73
2 minutes	46±19.47	31±19.08	61±23.64	30.67±9.81	51±12
*3 minutes**	26±11.36[a]	13.67±7.37[b]	48±13.75[abc]	10.33±3.51[acd]	47±10.58[abd]
*5 minutes**	17±7.55[e]	3.67±4.62[e]	13.67±5.77[e]	5±4.58[e]	69.67±2.31[e]

The CFU values are described as the survival rates that were compared with the negative control at 100%

*p <0.05 that the antifungal effect of CAMP for each trial time, the Kruskal-Wallis test

[a,b,c,d, and e] p <0.05 that comparison of the antifungal effect of CAMP for each condition, the Mann-Whitney U test

Different letters mean statistically significant differences($p<0.05$)

Data are presented as mean standard deviation

Table 2. Survival rates of *Malassezia pachydermatis* colony forming units (CFU) after cold atmospheric microwave plasma and chlorhexidine gluconate

	Treatment						
time	0.02% CHX	0.2% CHX	2% CHX	0.02% CHX + Plasma	0.2% CHX + Plasma	2% CHX + Plasma	Plasma
1 minute*	39.67 ± 13.01^{a}	5.67 ± 3.79^{ab}	0^{abc}	15.33 ± 11.59^{acd}	1 ± 1.73^{abde}	0^{abc}	36 ± 10^{bcde}
2 minutes*	28.67 ± 5.03^{a}	5 ± 4.58^{ab}	0^{abc}	19 ± 6^{abcd}	4.67 ± 4.73^{ade}	0^{abc}	30.67 ± 9.81^{bcde}
3 minutes*	27.33 ± 10.69^{a}	4.67 ± 4.04^{ab}	0^{ac}	19.33 ± 6^{bcd}	1.33 ± 2.31^{ade}	0^{ac}	10.33 ± 3.51^{acde}
5 minutes*	23.67 ± 13.45^{a}	3.67 ± 3.51^{ab}	0^{ac}	14.67 ± 9.29^{bcd}	1.33 ± 1.53^{ad}	0^{ac}	5 ± 4.58^{ac}

The CFU values are described as the survival rates that were compared with the negative control at 100%

*p <0.05 that the antifungal effect of CAMP for each trial time, the Kruskal-Wallis test

$^{a,b,c,d,\text{ and }e}p$ <0.05 that comparison of the antifungal effect of CAMP for each condition, the Mann-Whitney *U* test

Different letters mean statistically significant differences($p<0.05$)

Data are presented as mean standard deviation

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국문초록

*Malassezia pachydermatis*에 대한 저온 마이크로파 플라즈마의 항진균 작용

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Plasma는 고온 상태에서 물질이 이온화 된 상태로서 고체, 액체 및 기체 다음인 제 4의 물질 상태로 알려져 있다. 그러나 저온 마이크로파 플라즈마(Cold atmospheric microwave plasma; CAMP)는 40℃ 이하에서 plasma를 생성하여 의료 분야에서 세균, 곰팡이, 바이러스 등에 대한 살균을 주된 목적으로 사용되고 있다. *Malassezia pachydermatis*는 개의 알레르기성 피부염과 외이염을 악화시키고 말라세치아 피부염을 유발하는 주된 곰팡이균이다. 그러나 수의 분야에서 *M. pachydermatis*에 대한 CAMP의 항진균 작용에 대한 연구는 전무한 실정이다. 그렇기에 본 학위논문의 목적은 2020년 개의 귀에서 분리한

M. pachydermatis 균주에서의 CAMP에 대한 항진균작용 및 클로르헥시딘과의 상승 작용을 연구함에 있다.

2020년 외이염이 있는 개의 피부 및 귀에서 분리된 *M. pachydermatis* 균주를 대상으로 하였으며 CAMP에 대한 항진균 작용은 집락 형성 단위(Colony Forming Unit; CFU)를 이용하여 확인하였다. CAMP에 대한 클로르헥시딘과의 상승 작용은 이미 연구가 진행된 *M. pachydermatis* 균 배양액과 클로로헥시딘 용액의 혼합액에 CAMP를 적용하여 집락 형성 단위 (CFU)를 이용하여 확인하였다.

이 결과는 소동물 임상에서 2020년 개의 피부 및 귀에서 분리된 *M. pachydermatis* 균주에 대하여 CAMP가 대체로 뛰어난 항진균 작용을 나타내는 것으로 확인되었다. 먼저, plasma를 발생시키는 ignition 과정이 없이 CAMP를 적용하였던 음성대조군의 경우 항진균 작용이 없는 것으로 확인되었으며 ignition 과정은 CAMP의 *M. pachydermatis* 균주에 대한 항진균 작용에 필수적인 과정으로 확인되었다. 그리고 CAMP를 3분 이상 적용한 경우 CAMP의 *M. pachydermatis* 균주에 대한 항진균 작용이 통계적으로 유의하게 항진균 효과를 나타내는 것으로 확인되었다. 또한, CAMP를 3분 이상 적용하였을 때 플라즈마 출력 세기(W)가 클수록 CAMP의 *M. pachydermatis* 균주에 대한 항진균 작용이 통계적으로 유의하게 항진균 효과를 나타내는 것으로 확인되었으며 5분 적용 시에 *M. pachydermatis* 균주에 대한 생존율은 5% 전후로 확인되었다. *M. pachydermatis* 균주에 대한 CAMP의 항진균 작용이 확인함과 동시에 클로르헥시딘과의 상승 작용 또한

확인되었다. CAMP와 0.02%, 0.2% 클로르헥시딘을 각각 1, 2 분과 1분간 동시에 적용했을 경우, CAMP를 단독 적용했을 경우에 비해 *M. pachydermatis* 균주에 대한 생존율이 더 낮은 것으로 확인되었다.

이 결과는 소동물 임상에서 2020년 피부 및 귀에서 분리된 *M. pachydermatis* 균주에서의 CAMP에 대한 항진균 작용이 대체로 뛰어나다는 사실을 말해주고 있으며, 이미 항진균 효능이 입증된 국소제제와의 상승 효과 또한 있음을 말해주고 있다. 이는 말라세치아 피부염, 외이염, 알레르기성 피부염 등 피부 질환을 앓는 개에서 다수 검출되는 *M. pachydermatis* 균에 대한 새로운 치료 방안을 제시하고 국소제제와의 병용을 통한 상승 효과 또한 가능성이 있음을 시사하고 있다.

주요어: 개, 저온 마이크로파 플라즈마, 항진균 효과, *Malassezia pachydermatis*, 클로르헥시딘, 상승 효과

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