Effect of Different Shampoos and Contact Time on *Microsporum canis* Infected Hair: *In vitro* Model Study

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ABSTRACT

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Background: Tinea capitis is commonly caused by *Microsporum canis.* The standard treatment is systemic antifungal medications with adjunctive use of an antifungal shampoo. The current information about the effect of shampoos is limited.

Objectives: To compare the effects of different shampoos and contact times on *M. canis* infected hair.

Methods: This *in vitro* study was conducted at the Mycology Laboratory in a tertiary hospital, Thailand, in 2020. Hair taken from a child was disinfected and incubated with an *M. canis* colony for 15 days before divided into ten groups. Each of these samples was then mixed with distilled water, a commercial shampoo, a ketoconazole shampoo, a selenium sulfide shampoo, or a zinc pyrithione shampoo for either 2 or 5 minutes. Those samples and control-group samples were cultured for 7 days before fungal growth areas were collected and analyzed.

Results: At Day 3, the fungal colony growth areas on the samples mixed with commercial shampoo, ketoconazole shampoo, selenium sulfide shampoo, and zinc pyrithione shampoo were significantly smaller than that of the control. Moreover, the samples mixed with ketoconazole shampoo, selenium sulfide shampoo, and zinc pyrithione shampoo demonstrated a statistically different fungal-growth area from the control at Day 7. However, the duration of mixing resulted in similar areas of fungal growth for all samples.

Conclusions: Shampoos containing zinc pyrithione and commercial shampoos are alternative adjunctive treatments for tinea capitis arising from *M. canis.* In addition, contact with the shampoos for at least 5 minutes appears to be optimal.

Key words: Tinea capitis, *Microsporum canis,* zinc pyrithione shampoo, selenium sulfide

Background

Tinea capitis, commonly caused by *Microsporum canis*[1-3], is frequently detected in children[3,4]. Its standard treatment is systemic antifungal medications[1,2,5] using a ketoconazole or selenium sulfide shampoo as an adjunctive therapy[5]. An *in vitro* study established that azole shampoos could disinfect *M. canis* infected hair[6]. However, research on the effects of shampoos containing zinc pyrithione on *M. canis* infected hair, and investigations into the optimal contact time for the use of adjunctive shampoos for tinea capitis, is limited. Hence, we compared the effects of different shampoos and contact times on *M. canis* infected human hair.

Material and Methods

This *in vitro* study was conducted at the Mycology Laboratory in a tertiary hospital, Thailand, in 2020 after its approval by the

institution's review board. Locks of normal hair taken from a child were cut into 1-cm pieces before being disinfected and incubated with an *M. canis* colony for 15 days. This method was adapted from the standard hair perforation test[7]. The pieces were then divided into one control and ten experimental groups, all with equal amounts of hair. The hair samples for the control group were directly cultured in Sabouraud's dextrose agar with cycloheximide for seven days, without any liquid. As to the ten experimental groups, they were subdivided into five sets of pairs. Next, the following five liquids were added to the hair samples, with only one substance used for each pair:

1) distilled water;

2) a commercial shampoo (Siriraj Mild Shampoo - a non-antifungal, medicated-formulation shampoo; Siriraj Hospital, Bangkok, Thailand);

3) a 2% ketoconazole shampoo (Johnson and Johnson (Thailand) Ltd., Bangkok, Thailand);

4) a 2.5% selenium sulfide shampoo (Selson; Mentholatum Co. Inc., New York, USA); or

5) a zinc pyrithione shampoo (Head & Shoulders; Procter and Gamble (Singapore) Pte. Ltd., Singapore).

The liquid and hair samples were thoroughly combined using a vortex mixer. For each pair of liquid and hairpieces, one of the two sets was mixed for 2 minutes, while the other set was mixed for 5 minutes. After that, each of the ten experimental samples was cultured in Sabouraud's dextrose agar with cycloheximide. On Days 3 and 7, the colonies' areas were recorded using the software, ImageJ (version 1.8; National Institutes of Health, Bethesda, Maryland, USA). Specimens with contamination from other fungi were excluded from the study. Linear regression was employed to compare the effects of the different shampoos and contact times on the size of fungal colony growth areas. All statistical analyses were performed using SPSS Statistics for Windows, version 18.0 (SPSS Inc., Chicago, IL, USA).

Results

At Day 3, the areas of fungal colony growth on the hair samples mixed with commercial shampoo, ketoconazole shampoo, selenium sulfide shampoo, and zinc pyrithione shampoo were significantly smaller than that of the control sample ($p < 0.001$; Figure 1 and Table 1). Compared with the control-group sample, only the hair samples mixed with ketoconazole shampoo, selenium sulfide shampoo, and zinc pyrithione shampoo showed a statistically significant difference area of fungal growth at Day 7 ($p < 0.05$; Figure 2 and Table 1). On the other hand, the duration of mixing the shampoos and the hair samples (2 and 5 minutes) resulted in similar fungal growth; however, median area of

fungal growth of 2-minute mixing were smaller than 5 minute-mixing (Figure 1-2 and Table 1). A visual comparison of the fungal colony growth areas using ImageJ is presented in Figure 3.

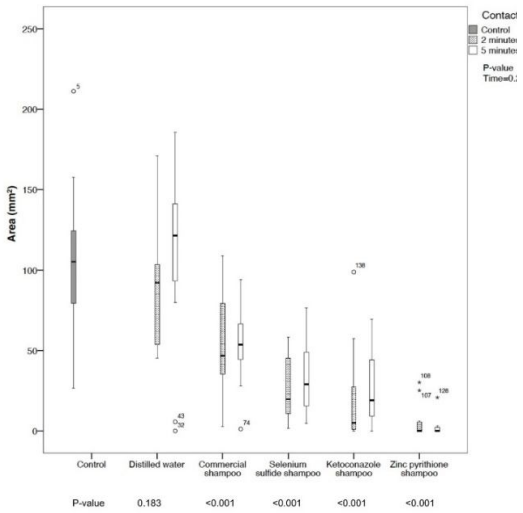

Figure 1 Median area of fungal growth on Day 3

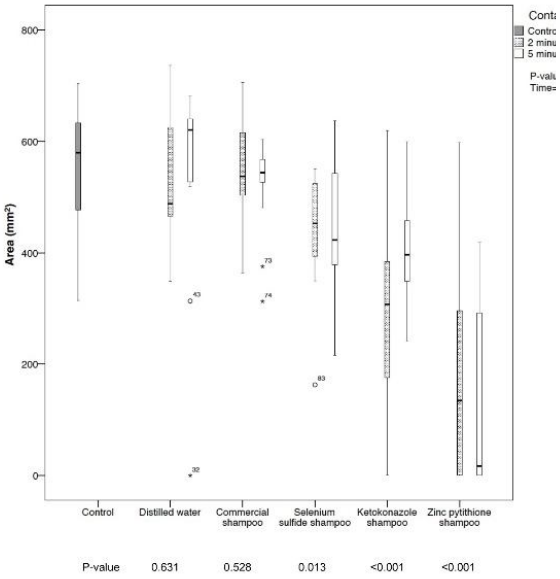

Figure 2 Median area of fungal growth on Day 7

Table 1 Median area of fungal growth for each group

	Contact time (minutes)	Median area of colony (IQR)/(mm^2)	
		Day 3	Day 7
Control	N/A	105.1 (78.6–126.9)	579.7 (427.2–640.7)
Distilled water	2	92.2 (52.6–104.7)	487.9 (461.7–628.3)
	5	121.5 (85.4–141.9)	621.0 (521.5–643.6)
Commercial shampoo	2	46.7 (35.1–85.6)	537.7 (502.4–642.1)
	5	53.7 (37.6–71.8)	543.8 (526.1–569.9)
Selenium sulfide shampoo	2	19.8 (9.0–46.9)	542.9 (386.3–524.5)
	5	30.0 (15.3–56.9)	423.2 (366.6–554.4)
Ketokonazole shampoo	2	5.0 (0.3–36.3)	307.5 (166.9–429.8)
	5	19.1 (7.8–44.5)	396.4 (349.0–463.4)
Zinc pyrithione shampoo	2	0 (0–5.5)	134.7 (0–354.9)
	5	0 (0–2.2)	17.0 (0–302.7)

Abbreviation: IQR, interquartile range

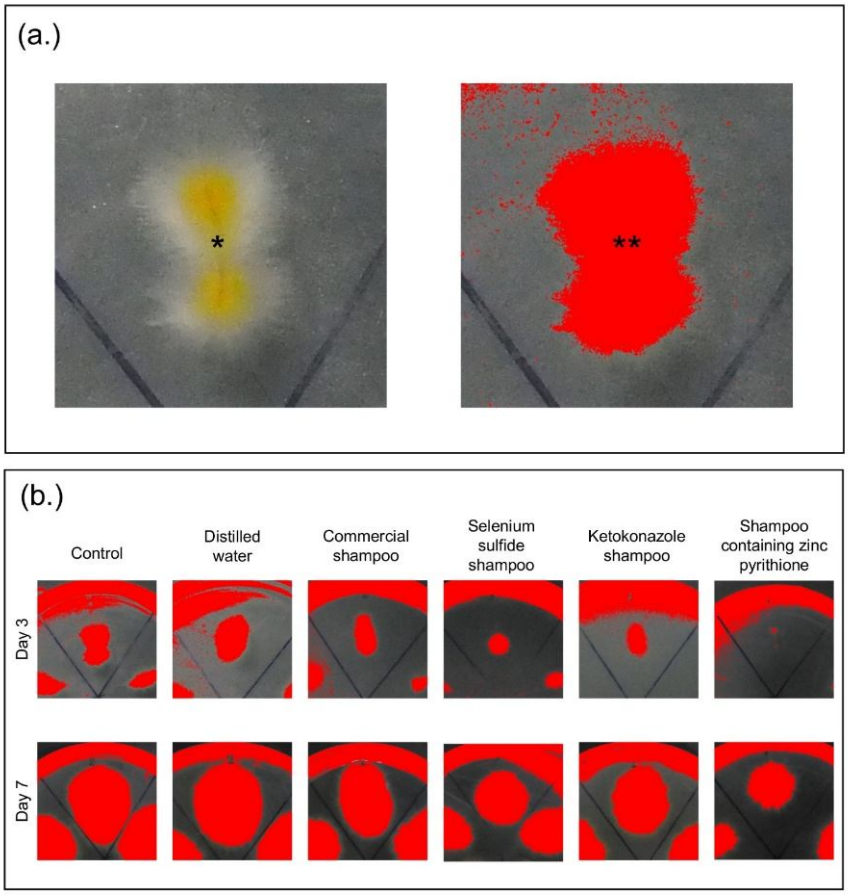

Figure 3 (a.) Fungal colony growth on fungal culture plate (*) and measurement of fungal colony growth using ImageJ (**) (b.) Areas of fungal colony growth using ImageJ

Discussion

This study shows that mixing *M. canis* infected hair with commercial shampoo, ketoconazole shampoo, selenium sulfide shampoo, and zinc pyrithione shampoo partially inhibited fungal growth. Therefore, the research findings support many national guidelines which recommend that the washing of infected hair with a shampoo should only be considered adjunctive therapy[1,2,8]. Instead, a systemic antifungal should be the mainstay of treatment[1,2].

Commercial shampoos can reduce fungal colonies' size on Day 3 as shampoo pH is alkaline, which is not suitable for fungal growth[9]. The *in vitro* growth of *Trichophyton rubrum* and other pathogens depends on having an initial optimum

pH for the cell culture medium of 4.0–5.0[9]. Furthermore, the surfactants in commercial shampoos (such as cocamidopropyl betaine and sodium laureth sulfate) enhance their cleansing properties and may affect the degree of fungal growth[10]. A limited study was conducted to demonstrate the efficacy of using general shampoos to inhibit fungal growth (a more accessible approach for patients than obtaining relatively expensive, specialized shampoos). This study showed that commercial shampoo could reduce fungal growth. However, as the growth rebounded on Day 7, washing with a general shampoo to inhibit fungal growth should be repeated every three days.

Our investigation found that zinc pyrithione shampoo demonstrated promising results in inhibiting *M. canis* growth on Days 3 and 7. An earlier *in vitro* study showed that zinc pyrithione inhibited fungal growth by increasing the cellular levels of copper and damaged iron-sulfur clusters of proteins essential for fungal metabolism[11]. Ketoconazole shampoo and selenium sulfide shampoo, widely recognized as adjunctive treatments for tinea capitis[1,2,5,8], also resulted in a small fungal growth on Days 3 and 7. Moreover, several *in vivo* studies in children revealed the benefits of ketoconazole shampoo and selenium sulfide shampoo for treating tinea capitis[8,12,13]. These shampoos could be used to decrease fungal growth in patients with an *M. canis* hair infection.

Mixing the *M. canis* infected hair with distilled water or the 5 studied shampoos for 2 minutes versus 5 minutes produced the reductions in the fungal colonies' areas in our study. Two-minute mixing could reduce the growth of fungus more than 5-minute mixing; however, we could not conclude that 2 minutes were enough for applying shampoo at infected hairs since the study sample size were too small. The recommended guideline for tinea capitis has suggested at least 5 minutes to apply at hairs[14]. Thus, our study supported that minutes' timing could be optimal for washing hair at least 5 minutes with the studied shampoos as an adjunctive treatment for tinea capitis arising from *M. canis*.

This work has several limitations. Firstly, as many dermatophytes can cause tinea capitis[1,2,4], the study only used *M. canis* as a representative agent for the experiment. Research on other dermatophytes that can cause tinea capitis is required. Besides, the number of samples in our investigation was small; nevertheless, the results may guide further *in vivo* research. An *in vivo* study on humans utilizing a large cohort of participants with tinea capitis would more fully elucidate the relative efficacies of commercial shampoos and zinc pyrithione shampoo for tinea capitis.

Conclusion

Zinc pyrithione shampoo and commercial shampoos might be considered alternatives for the adjunctive treatment of tinea capitis stemming from *M. canis.* Zinc pyrithione shampoo had comparable effects with ketoconazole shampoo and selenium sulfide shampoo. Shampooing the hair for at least 5 minutes could be the optimal contact time for the adjunctive treatment of tinea capitis from *M. canis.*

Conflict of interes

None declared.

Finding Statement

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