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Factors determining the antidandruff effect of Climbazole in a shampoo formulation

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Summary

Dandruff is a persistent and annoying common condition affecting the scalp. It is also a challenging disease/disorder for management. This study reports the effect of shampoo ingredients on the anti-dandruff effect of climbazole in a shampoo formulation. The results indicate that the interaction between various ingredients greatly contribute to the antidandruff activity of climbazole. The antidandruff activity of climbazole was superior in SLES when compared to other surfactants used in the study.

Riassunto

La forfora è una disfunzione comune e fastidiosa che colpisce il cuoio capelluto. Il come affrontare questa disfunzione/patologia rappresenta anche una sfida.
Questo studio riporta gli effetti di alcuni ingredienti di uso cosmetico sull'attività antiforfora esercitata dal climbazolo inserito negli shampoo.
L'attività di questo antifungino si è dimostrata più elevata con l'uso del Sodio Lauril Etere Solfato (SLES) rispetto ad altri tensioattivi.

INTRODUCTION

Dandruff is one of the most commercially exploited skin diseases by the personal care industry all over the world (1). The lipophilic yeast of genus *Malassezia* is implicated in causing dandruff and the most common species being *M. globosa M. restricta and M. furfur* (2). There are several factors in dandruff formation such as excess sebum production, dryness of scalp due to continuous use of shampoos/ hair conditioners, excessive combing etc. (1,3).

The etiology and various predisposing factors of the disease are still unknown, dandruff remains a challenge despite availability of therapeutic options.

Shampoo is one of the best hair cleansers and its use dates back to 1972 (4). Antidandruff shampoo is a very complex chemical system that contains primary, secondary, amphoteric & anionic surfactants besides conditioners, detangling agents, hair softeners and antidandruff agents. Performance of an antidandruff agent in such a complex system is always an area of concern. The pH sensitivity, solubility, availability and substantive deposition of the antidandruff agents on the scalp during shampoo wash are the key factors determining the activity of the antidandruff agents. Hence, the formulation of an antidandruff shampoo must fit into the above matrix of understanding to enable it to be effective against the causative organisms.

Varieties of antidandruff agents are used widely in various antidandruff preparations such as climbazole, zinc pyrithione, octopirox, ketoconazole, selenium sulphide, coal tar etc. Among these, climbazole is one of the most popular antidandruff agents. It is an imidazole antifungal with well-proven safety data (5). The European Commission's Scientific Committee on Consumer Products is of the opinion that the use of climbazole in rinse-off hair cosmetics including its use as antidandruff active ingredient up to a maximum concentration of 2% does not pose any risk to the health of consumer and hence it is an ideal candidate for antidandruff shampoos. Further, climbazole can be used in both transparent and opaque preparations without much formulation and stability challenges. The present study reports the various factors affecting the anti-fungal activity of climbazole in an antidandruff shampoo against different species of *Malassezia*.

MATERIALS AND METHODS

Antifungal testing

Antifungal testing of climbazole was done using standard procedure (6). Five strains each of *M.globosa, M.restricta and M.furfur* recovered from human scalp were used in the study. The strains were maintained in Dixon agar with periodic subculture.

Climbazole was solublized in 5% DMSO and a stock solution of 10mg/ml was prepared. Broth dilution test was performed for antifungal assay (7). Fungal cells adjusted to the absorbance of 0.6 at 450nm were used as inoculum.

Sabouraud's dextrose agar with Tween 60 was used as test medium. The test plates were incubated at 26°C for 7 days.

The minimum inhibitory concentration (MIC) was determined as per standard procedure (7).

Ten percent (10%) solutions of various surfactants such as sodium lauryl ether sulfate (SLES), cocomonoethanolamide (CMEA), cocamidopropyl betaine (CAPB), cocodiethanol amide (CDEA) were prepared. Climbazole at a concentration of 2mg/ml was added to these preparations as stock and were used for testing the activity against test organisms at various concentrations. 2mg/ml solution of climbazole in 10% DMSO was used as control. Above preparations without climbazole was also used separately for testing the effect of these agents on the test orga-

nisms. A similar procedure to study the effect of climbazole in silicon oil and isopropyl palmitate was also done.

A formulation of shampoo prepared with 1% climbazole was tested for its activity against different species of *Malassezia*. A shampoo (brand not disclosed to avoid any commercial implication) with 1% climbazole was procured from the market and tested for its activity. The pH of the test shampoo formulation was adjusted to the pH of the market sample to achieve uniformity of results.

Methylene blue reductase test

This test was done to establish the contact time of the formulation vs. % kill of *Candida albicans* cells. The yeast cells (10^4 *cfu*) were incubated with 10% solution of various formulations for 2, 5 and 10 minutes. After incubation, the tubes were centrifuged, washed with saline, stained with methylene blue and examined under microscope (8). Ten fields at random were chosen and the number of stained vs. unstained cells were counted on relative abundant basis and the % kill effect of the formulation vs. the contact time was established.

RESULTS

Among the three different species of *Malassezia* tested, *M. globosa* was observed to be relatively more susceptible to climbazole when compared to other two species viz., *M. furfur* and *M. restricta* (Table 1). The MIC of climbazole was noted to be in the range of 62.5 – 125 μg/ml.

The efficacy of climbazole was tested in different ingredients that are commonly used for formulating an antidandruff shampoo.

The shampoo ingredients were prepared as 10% solution. Using the above solutions of each of the ingredients, a stock of 2mg/ml of climbazole was prepared.

The activity of climbazole in different shampoo ingredients was tested.

The activity of climbazole was superior in SLES when compared to other surfactants tested. Similarly the activity of climbazole was least in CMEA. The susceptibility pattern of different species of Malassezia was also different (Table 2). Activity of climbazole was lower in CDEA when compared to CAPB. Interestingly, the activity of climbazole in dimethicone and isopropyl palmitate was relatively stable.

None of the shampoo ingredients (10% solution) on 'as is basis' showed any activity against all the species of *Malassezia* tested.

The MIC of climbazole in 5% DMSO was in the range of 31.25μg/ml for *M. furfur* and *M. restricta* and one level higher (31.25μg/ml) for *M. globosa*. The activity of climbazole in 5% DMSO was taken as reference to study the interfering role of various shampoo ingredients in the activity of climbazole (Table II).

TABLE I

MIC of climbazole on Malassezia species on 'as is basis'

Species	No. of strains	Concentration in μg/ml					
		1000	500	250	125	62.5	31.25
M.furfur	5	-	-	-	-	+	+
M.globosa	5	-	-	-	-	-	+
M.restricta	5	-	-	-	-	+	+

'-' = Absence of growth of the organism, '+' = Presence of growth

TABLE II

MIC of climbazole in different shampoo ingredients (the ingredients were prepared as 10% solution in water, except dimethicone)

Test materials	Susceptibility of different species / μg/ml		
	M.furfur	***M.restricta***	***M.globosa***
SLES	NA	NA	NA
SLES+climbazole	125	125	125
CMEA	NA	NA	NA
CMEA+climbazole	1000	1000	1000
CDEA	NA	NA	NA
CDEA+climbazole	500	500	500
CAPB	NA	NA	NA
CAPB+climbazole	250	250	250
Dimethicone	NA	NA	NA
Dimethicone+climbazole	31.25	62.5	62.5
Isopropyl palmitate	NA	NA	NA
Isopropyl palmitate + climbazole	62.5	62.5	62.5
5% DMSO + climbazole	31.25	31.25	62.5

Based on the interfering role of various shampoo ingredients on the activity of climbazole, an antidandruff shampoo formulation was done with climbazole at 1% level. Similarly, a market antidandruff shampoo with climbazole at 1% level was procured.

The market sample and the formulated shampoo were tested for the activity against different species of *Malassezia*.

The formulated antidandruff shampoo exhibited activity at 125μg/ml against different species of *Malassezia*. Activity of the market shampoo was at 1000μg/ml.

When the pH of the market shampoo was adjusted to acidic, the shampoo exhibited relatively superior activity (Table III).

Minimum contact time required to cause total death of the yeast cells was studied by methylene blue reductase test using *C. albicans*. Near to total death of all yeast cells was observed when the test formulation of shampoo was treated with yeast cells for 5 minutes. Only 50% death of yeast cells was observed even after 10 minutes of treatment of yeast cells with the market shampoo (Table IV).

TABLE III

MIC of controlled shampoo formulation and a market shampoo with climbazole at 1% level

Test samples	MIC of 1% climbazole / μg/ml		
	M. restricta	***M. globosa***	***M. furfur***
Controlled formulation	125	125	125
Market sample	1000	1000	1000
pH adjusted market sample	500	500	500

TABLE IV

Contact time vs. % death of yeast cells

Test products	Contact time/ % death (% stained vs. unstained yeast cells)		
	2min	5min	10min
Controlled formulation	70	100	100
Market sample	20	40	50

DISCUSSION

The present study revealed several interesting aspects on the role of different shampoo ingredients in the antidandruff activity of climbazole. When the role of different shampoo ingredients on the antidandruff effect of climbazole was tested individually, we observed that CMEA and CDEA significantly brought down the activity of climbazole. Interestingly, SLES and CAPB did not reduce the anti-dandruff activity of climbazole significantly when compared to control. Based on the above findings, we hypothesize that an effective antidandruff shampoo should contain less level of CMEA and CDEA when climbazole is used as antidandruff agent. Accordingly, an anti-dandruff shampoo was formulated with CMEA and CDEA at 2% level and climbazole at 1%. The shampoo formulation developed was tested along with a market sample with climbazole at 1% level. Interestingly, we observed the formulation made by us exhibited superior activity when compared to the market sample.

The second postulate we framed was to establish the role of pH in the activity of climbazole. For this purpose, we adjusted the pH of our shampoo from 5-7.4. Although the acidic pH was found to enhance the activity of climbazole (9), the role pH was not as significant as various ingredients used for formulating a shampoo. It is usual that the level of surfactants used for antidandruff shampoo are relatively higher than the normal shampoo. Higher level of use of surfactants in antidandruff shampoo is expected to play a role in effective clearing of scalp cells. However, our study has proved that shampoo with lower level of surfactants is most effective when climbazole is used as antidandruff agent. The data of the present study however cannot be extrapolated for other antidandruff ingredients and hence warrants a detailed study.

To simulate a likely *in vivo* activity, we studied the contact time of the shampoo vs. % death of *C. albicans*. Near complete death of yeast cells was found within 5minutes of contact time with our formulated shampoo as against 50% death even after 10 minutes contact time with the market shampoo.

The above findings suggest that despite the same level of climbazole in both shampoos, the interplay of various shampoo ingredients greatly contribute to the antidandruff activity of climbazole. Findings of the present study clearly reveal that formulation of any 'functional' personal care product needs a proper understanding of all the constituent ingredients and their interactions with each other. It is widely believed in personal care industry that higher the number of ingredients; better is the effect. But our study establishes that simpler the formulation containing synergistic ingredients better is the delivery of functional benefit.

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