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ORIGINAL ARTICLE

Cosmetic antidandruff shampoo found far more effective than ketokonazole shampoo: probable contribution of thermal mineral water

Uno shampoo antiforfora cosmetico si è rivelato molto più efficace di uno shampoo al ketoconazolo: probabile contributo dell'acqua minerale termale

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Abstract - Riassunto

BACKGROUND: There is evidence suggesting the role of *Malassezia* species in the pathogenesis of dandruff and seborrheic dermatitis (D/SD). They were shown to have lipase activity, whose metabolites trigger inflammatory response inducing keratinocytes to produce pro-inflammatory cytokines. Considering the inflammatory features of the disorders suggested, we conducted a clinical trial on D/SD patients, in order to reveal the efficacy of a specially prepared cosmetic shampoo manufactured with a thermal mineral water, which has authenticated *in-vitro* anti-inflammatory effects.
METHODS: The shampoo which was manufactured with a thermal mineral water; comprised a combination of antifungal agents, piroctone olamine and zinc pyrithione, within the EU cosmetic regulation limits. One-week clinical trial on 30 subjects with D/SD was randomized, controlled and double-blind. A commercial medicinal shampoo containing ketokonazole as active ingredient was used as reference. The symptoms/signs of each subject were recorded according to a semi-quantitative severity scale, and close-up photographs were taken at the beginning and at the end of the study.
RESULTS: Although the results were favorable and statistically significant in both groups, contrary to expectations, the cosmetic shampoo was found to be apparently more effective than the medicinal shampoo.
CONCLUSIONS: The superiority of the cosmetic shampoo can be attributed to the anti-inflammatory features of thermal mineral water in the formulation, which are probably due to its high mineral content, especially sulfur, zinc and boric acid. In this context, the thermal mineral water may be considered as an additional active ingredient.

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KEY WORDS: Mineral waters; Dandruff; Dermatitis, seborrheic.

OBIETTIVO: *Numerosi studi scientifici suggeriscono che la specie Malassezia abbia un ruolo importante nella patogenesi della forfora e della dermatite seborroica (F/DS). È stato determinato che la Malassezia ha un'attività lipasica i cui metaboliti innescano una risposta infiammatoria che induce i cheratociti a produrre citochine postinfiammatorie. Considerando le caratteristiche infiammatorie dei disturbi suggeriti, abbiamo condotto una sperimentazione clinica su pazienti affetti da F/DS per determinare l'efficacia di uno shampoo cosmetico preparato appositamente con acqua minerale termale, che ha effetti antinfiammatori in vitro dimostrati.*
METODI: *Lo shampoo preparato con acqua minerale termale conteneva una combinazione di agenti antimicotici, piroctone olamina e zinco piritione, nel rispetto deli limiti del regolamento UE sui cosmetici. È stata condotta una sperimentazione clinica randomizzata, controllata e in doppio cieco su 30 soggetti affetti da F/DS. Come riferimento è stato utilizzato uno shampoo medicinale commerciale contenente come principio attivo il ketokonazolo. I sintomi di ciascun soggetto sono stati registrati secondo una scala di gravità semiquantitativa e sono state scattate fotografie all'inizio e alla fine dello studio.*
RISULTATI: *Sebbene i risultati siano stati favorevoli e statisticamente significativi in entrambi i gruppi, contrariamente alle aspettative lo shampoo cosmetico si è rivelato apparentemente più efficace dello shampoo medicinale.*
CONCLUSIONI: *La superiorità dello shampoo cosmetico può essere attribuita alle caratteristiche antinfiammatorie dell'acqua minerale termale utilizzata nella formulazione, che sono probabilmente dovute al suo alto contenuto minerale, in particolare zolfo, zinco e acido borico. In questo contesto, l'acqua minerale termale può essere considerata un principio attivo aggiuntivo.*

Dandruff and seborrheic dermatitis (D/SD) are disorders with many common features, also responding to similar treatments. Dandruff is generally restricted to scalp and comprises flaking and sometimes itchy skin without inflammation. Seborrheic dermatitis (SD) affects wider seborrheic regions like face, upper chest and back, as well as scalp; causing squama, crust, erythema and pruritus, generally with marked inflammation. There is evidence suggesting the role of *Malassezia* species (sp.) in the pathogenesis of D/SD.[1] Incidence of SD is 1-3% of the general adult population.[2, 3] Men are affected more frequently than women in all age groups, suggesting a role of androgens in the pathogenesis.[1, 2] Dandruff is much more common than SD, and affects approximately 50% of the general adult population worldwide. Like in SD, it is also more prevalent in males than females.[4, 5] Dandruff starts at puberty, reaches peak incidence and severity in twenties, and becomes less prevalent among people over fifty.[5] Besides physical discomfort such as itching, dandruff is socially embarrassing and negatively impacts patient's self-esteem.[4] Women, younger patients, and subjects with higher educational level are more affected emotionally.[6] In general, both the disorders present common features histologically. Epidermal hyperplasia, parakeratosis, and Malassezia yeasts surrounding the parakeratotic cells may be seen in both conditions.[5] But, although inflammatory cells such as lymphocytes and natural killer cells may be present in great numbers in SD, dandruff shows subtle or no inflammation. These outcomes support the modality that D/SD are manifestations of the same disease with different severities.[7] Primarily symptomatic relief is targeted in treatment of D/SD. The ultimate success of therapy is to maintain remission and preclude exacerbations in the long-term. The most widely used agents in treatment are topical antifungal and anti-inflammatory preparations.[8] Systemic therapy is needed only in widespread lesions and in cases that do not respond to topical treatment.[1, 2] In this study, we formulated and clinically tested the efficacy and safety of a cosmetic shampoo containing thermal mineral water which was reported to have anti-inflammatory effects in the cell cultures.[9] The formulation also contained a combination of antifungal agents, piroctone olamine and zinc pyrithione, within the EU cosmetic regulation limits. The reference formulation was a medicinal ketokonazole shampoo available commercially.

Materials and methods

The sample consisted of otherwise healthy 15 male and 15 female Caucasian subjects enrolled in the trial with written informed consent, between 18 and 52 years old, suffering from D/SD. The subjects were included in the trial by the dermatologist, among the outpatient referrers for D/SD, on the basis of the inclusion/exclusion criteria: subjects were not using any topical or systemic medications at least for two weeks; no scalp or hair disease except the trial indications; no hypersensitivity history and none of them were pregnant or in lactation period. The study protocol was approved by the local (institutional) ethical committee and all the trial procedures were performed in rapport with the ethical principles established for medical research (Helsinki Declaration of World Medical Association 1964, and amendments). After the situation assessments of scalp and hair, symptoms/signs of the patients were recorded according to four grade semi-quantitative severity scale for squama, crust, and erythema by the dermatologist. Grading was performed as, 0: null, 1: mild, 2: moderate, 3: severe. The evaluation of pruritus was performed according to self-declaration by the patients, in consistence with the same severity scale.

The active ingredients and excipients of the cosmetic shampoo (Antidandruff Shampoo) and the medicinal shampoo (ketokonazole shampoo) are depicted in Table I. The thermal mineral water used in the Antidandruff Shampoo was from Bolu Göynük, a province in Turkey. The *in-vitro* effects and its chemical composition were reported previously.[9] The chemical analysis of Bolu Göynük thermal mineral water (BTW) is presented in Table II. Although the other constituents in the list of excipients in either shampoo differ to some extent, all are far from being at concentrations to have any effect against D/SD and formulated for the inner consistency and user acceptability of the products.

Table I.—*The ingredient lists of antidandruff shampoo and ketokonazole shampoo.*

Variables	Antidandruff shampoo	Ketokonazole shampoo
Actives	*Piroctone olamine 0.75% (w/w)* *Zinc pyrithione 1% (w/w)*	*Ketokonazole 2% (w/w)*
Other Ingredients	*Aqua (Bolu Göynük natural mineral water)* magnesium laureth sulfate, disodium laureth sulfosuccinate, cocamidopropyl betaine, glycerin, decyl glucoside, acrylates crosspolymer-4, polysorbate 20, diazolidinyl urea, panthenol, salicylic acid, glycolic acid, niacinamide, polyquaternium-10, tetrasodium EDTA, sodium benzoate, potassium sorbate, mel, perfume/fragrance	*Aqua (purified water)* Sodium laureth sulfate, disodium laureth sulfosuccinate, sodium chloride,benzyl alcohol, citric acid monohydrate, laureth-2, quaternium-15, tetrasodium EDTA, polyquaternium-7, dexpanthenol,sodium hydroxide, erythrosine (E127), perfume/fragrance

Table II.—*Chemical analysis of Bolu Göynük thermal mineral water (BTW).*

Variables	Values
pH	9.25
Temperature	25 °C
Total mineralization	524 mg/L
Conductivity	402 µS/cm
Sodium, Na^{+}	124.146 mg/L
Magnesium, Mg^{2+}	0.608 mg/L
Calcium, Ca^{2+}	0.932 mg/L
Iron, Fe^{2+}	0.050 mg/L
Chloride, Cl^{-}	34.563 mg/L
Fluoride, F^{-}	8.900 mg/L
Bromide, Br^{-}	1.140 mg/L
Iodide, I^{-1}	0.154 mg/L
Bicarbonate, HCO_3^{-}	225.700 mg/L
Sulfur, S^{2-}	1.278 mg/L
Silicic acid, H_2SiO_3	63.680 mg/L
Boric acid, HBO_2	51.517 mg/L
Cadmium, Cd^{2+}	0.0016 mg/L
Chromium, Cr^{3+}	0.030 mg/L
Zinc, Zn^{2+}	0.100 mg/L
Molybdenium, Mo^{6+}	0.020 mg/L

Each patient was given a 100-cc uniform bottle with a randomly assigned specific number on it, which was blind for the dermatologist, prefilled with either antidandruff or ketokonazole shampoo. The subjects were informed about the use of the product as application a palm sized amount on wet hair every other day, three times a week, waiting for 3-4 minutes after foaming and then rinsing well. After one week, the patients were again evaluated according to the same severity scale. Also, digital close-up photographs of the scalp and hair of each patient were taken both before and after the treatment.

Statistical analysis

Gender distribution, age of the patients, and the disease durations until the trial are depicted for each shampoo group and all patients in Table III. Student's *t*-test (two-tailed, paired) was used for statistical analyses.

Results

Products were well tolerated, and no adverse effects were recorded during the study. Both of the products revealed a satisfactory outcome. The graphic presentations of the changes in scores of symptoms/signs after three applications in antidandruff and ketokonazole shampoo groups are shown in Figure 1 and Figure 2 respectively. Although the results are favorable and statistically significant in both groups, in terms of P values, antidandruff shampoo set

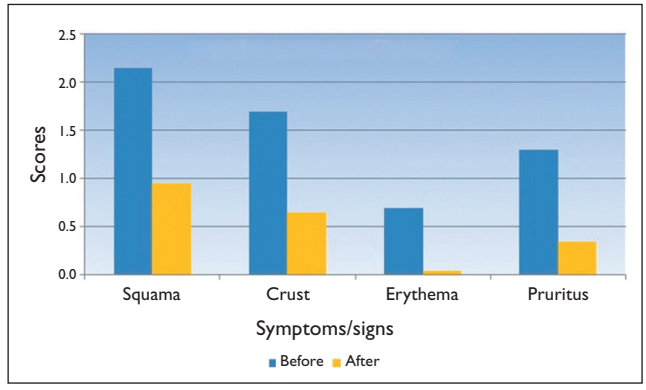

Figure 1.—Before and after scores of symptoms/signs in antidandruff shampoo group.

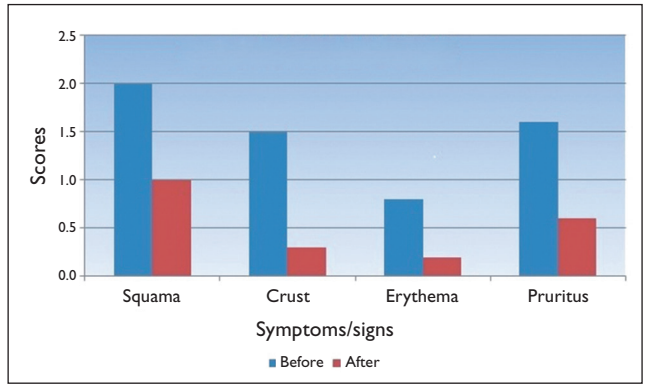

Figure 2.—Before and after scores of symptoms/signs in ketokonazole shampoo group.

Table III.—*Demographic data and the disease durations of the patients.*

Shampoo	Number	Gender	Age		Disease duration	
			Mean±SD	Median	Mean±SD	Median
Antidandruff shampoo	20	9 M, 11F	25.55±8.39	23.5	4.50±3.32	3.5
Ketokonazole shampoo	10	6 M, 4F	25.00±5.38	25.5	3.60±3.72	1.5
Total	30	15M, 15F	25.37±7.53	24	4.20±3.36	3
M: male; F: female; SD: standard deviation.						

Table IV.—*The P values referring to the comparison of before* vs. *after scores of symptoms/signs for each shampoo group.*

Shampoo	Squama	Crust	Erythema	Pruritus
Antidandruff shampoo	P<0.0001	P<0.0001	P<0.0001	P<0.0001
Ketokonazole shampoo	P=0.0011	P=0.0011	P=0.0239	P=0.0038

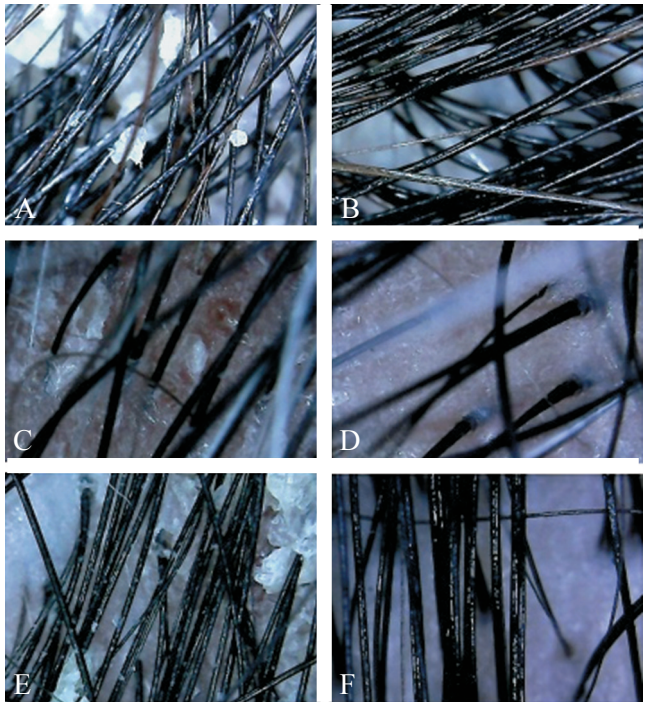

Figure 3.—Before (A, C, E) and after (B, D, F) close-up photographs of some patients in antidandruff Shampoo group, with various severity scores.

forth an obvious supremacy. The P values pertaining to the comparison of before *vs.* after scores of symptoms/signs for each shampoo group are presented in Table IV. In Figure 3, it is shown before and after close-up photographs of some patients in antidandruff shampoo group, with various severity scores.

Discussion

Malassezia are lipophilic yeasts that are found mainly on seborrheic regions of the body.[1, 8] They were shown to have lipase activity, which hydrolyze human sebum triglycerides and release unsaturated fatty acids such as oleic and arachidonic acids.[1] These metabolites lead to disrupted epidermal barrier function and commence inflammatory response inducing keratinocytes to produce pro-inflammatory cytokines such as IL-1α, IL-6, IL-8 and TNF-α, thus exacerbating the inflammatory response.[7, 10] Despite the proven role of *Malessezia sp*. in D/SD, they were also detected on normal skin of majority of healthy adults as commensal organisms.[1, 8] Also, although D/SD have an intense time/period correlation with sebaceous gland activity in lifetime,[1-3] the patients may have normal sebum production, and individuals with excessive sebum production sometimes do not develop D/SD. These findings suggest a strong evidence of individual predispositions and host interactions with *Malassezia* yeasts in the pathogenesis of D/SD. Disrupted epidermal barrier function due to genetic predisposition, and excessive or altered sebum composition provide a suitable medium for *Malassezia* colonization. Impaired epidermal barrier function facilitates entry of *Malassezia sp*. and their metabolites to the tissue. These metabolites irritate the epidermis and trigger host's immune response, and the revealed inflammatory reaction further disarrays epidermal differentiation. Pruritus and subsequent scratching may damage the barrier even further, leading to cycles of immune stimulation, and further barrier disruption.[1]

Antifungal agents are the basis of antiseborrheic therapy, usually in the azole form. Ketokonazole is one of these agents. It acts by inhibiting ergosterol synthesis, an important component of the fungal cell wall, via interference with the fungal cytochrome P-450 system. This causes an increase in the production of sterol precursors, a fungistatic process that precludes the fungus from growing or reproducing.[11] Another antifungal, piroctone olamine is a pyridine derivative. It is frequently used in many cosmetic products such as antiseborrheic shampoos and is known to have a fungicidal effect. Piroctone olamine penetrates inside the cell and forms complexes with iron ions, inhibiting fungal energy metabolism in mitochondria.[12] Zinc pyrithione is an antimicrobial active material that has been used for decades in cosmetic products to alleviate the symptoms of D/SD.[13] Zinc pyrithione inhibits fungal growth by causing increased intracellular copper levels and deactivating iron-sulfur cluster containing proteins, which are essential for fungal metabolism.[14]

In our study, Antidandruff Shampoo which involved moderate concentrations of piroctone olamine and zinc pyrithione, as allowed for cosmetic products, presented an apparent superiority against a medicinal shampoo whose active ingredient was ketokonazole with maximal allowed concentration. Although both shampoos generated statistically significant results as anticipated, this high level difference between the two groups was an unexpected outcome, which is most probably due to the anti-inflammatory properties of BTW used in the manufacturing process of antidandruff shampoo. In the study to reveal the anti-inflammatory effect of BTW, it was reported that it caused significant downregulation of IL-1α, TNF-α and vascular endothelial growth factor (VEGF) gene expres-

sions when incubated with a human keratinocyte cell line (HaCaT cells).[9] Normal human epidermis is an efficient source of biologically active IL-1α. Keratinocytes both synthesize this cytokine and respond to it via cell surface receptors, suggesting that the IL-1 system has an important function in epidermal physio-pathology.[15-17] Dysregulation of TNF has been presented in a wide variety of inflammatory diseases.[18] TNF-α, synthesized in epidermal keratinocytes, was shown to be a very potent inhibitor of follicle proliferation.[19] Intradermal injection of TNF-α caused a significant high number of apoptotic cells in the epidermis.[20] *In-vitro* studies revealed that TNF-α together with IL-1α, cause vacuolisation of matrix cells in the follicle bulb, disorganization of follicular melanocytes, and abnormal differentiation and keratinization of the precortical cells and the inner root sheath of the hair fiber.[19] Besides D/SD, these two cytokines are also thought to have important roles in the pathogenesis of acne, which is an inflammatory disorder closely related with sebaceous gland dysfunction.[21, 22] VEGF is produced by different types of cells and has a major role in both physiological and pathological angiogenesis. It is a strong mitotic and chemotactic factor for the endothelial cells, stimulating formation of new vessels. It also increases the endothelium permeability of the blood vessels, which facilitates the progression of inflammatory reactions, boosting the formation of oedema and leukocyte migration to the inflammation site. Its role in the pathogenesis of various inflammatory skin disorders has been reported.[23]

The above mentioned *in-vitro* anti-inflammatory effects of BTW may be partly due to its relatively higher sulfur content.[9] Sulfur, which is found as H_2S in thermal mineral waters, has well known anti-inflammatory and anti-oxidative effects in HaCaT cells. Antibacterial, antifungal and keratolytic properties of sulfur have enabled its use for the treatment of various skin diseases. Within the epidermis, H_2S is transformed to sulfur and later by the interaction with free oxygen radicals, it can be transformed to pentathionic acid, which may explain the antibacterial and antifungal properties of sulfurous mineral waters.[24] On the other hand, zinc and boric acid ingredients of BTW might also be partly responsible for the suppression of pro-inflammatory cytokines. Anti-inflammatory and antioxidant properties of zinc have been widely documented.[25, 26] Boric acid as a component of thermal mineral waters was shown to act on keratinocytes as a wound healing agent.[27] Therefore BTW, having high boric acid concentration, can be a promising candidate for the repairment of disrupted epidermal barrier function in D/SD.

These highly significant results obtained in a relatively short period in a small sample, also suggest performing larger scale clinical trials with products prepared using BTW against some other inflammatory conditions of the skin besides D/SD; such as acne, psoriasis, or atopic dermatitis.

Conclusions

In addition to classical antifungal active ingredients, the efficacy of cosmetic preparations against D/SD can be augmented by the use of thermal mineral waters in their formulations. In this context, the thermal mineral water, which is also the major component of the product, may be considered as a new active ingredient.

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