Three Etiologic Facets of Dandruff and Seborrheic Dermatitis: *Malassezia* Fungi, Sebaceous Lipids, and Individual Sensitivity

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Application of new molecular and biochemical tools has greatly increased our understanding of the organisms, mechanisms, and treatments of dandruff and seborrheic dermatitis. Dandruff results from at least three etiologic factors: *Malassezia* fungi, sebaceous secretions, and individual sensitivity. While *Malassezia* (formerly *P. ovale*) has long been a suspected cause, implicated by its presence on skin and lipophylic nature, lack of correlation between *Malassezia* number and the presence and severity of dandruff has remained perplexing. We have previously identified the *Malassezia* species correlating to dandruff and seborrheic dermatitis. In this report, we show that dandruff is mediated by *Malassezia* metabolites, specifically irritating free fatty acids released from sebaceous triglycerides. Investigation of the toxic *Malassezia* free fatty acid metabolites (represented by oleic acid) reveals the component of individual susceptibility. *Malassezia* metabolism results in increased levels of scalp free fatty acids. Of the three etiologic factors implicated in dandruff, *Malassezia*, sebaceous triglycerides, and individual susceptibility, *Malassezia* are the easiest to control. Pyrithione zinc kills *Malassezia* and all other fungi, and is highly effective against the *Malassezia* species actually found on scalp. Reduction in fungi reduces free fatty acids, thereby reducing scalp flaking and itch.

Key words: dandruff/individual susceptibility/*Malassezia*/microflora/sebaceous gland/seborrheic dermatitis/sebum
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Application of new molecular and biochemical tools has greatly increased our understanding of the organisms, mechanisms, and treatments of dandruff and seborrheic dermatitis. Dandruff results from at least three etiologic factors: *Malassezia* fungi, sebaceous secretions, and individual sensitivity. Although *Malassezia* yeasts (formerly *Pityrosporum ovale*) have long been a suspected cause, implicated by their presence on the skin and their lipophilic nature, lack of correlation between *Malassezia* number and the presence and severity of dandruff has remained perplexing. Significant recent data indicate a causal relationship between *Malassezia* and dandruff: (1) dandruff and seborrheic dermatitis (D/SD) can be effectively treated with a wide range of material types, from zinc salts, selenium salts, and glycols to highly specific azoles, with the only known commonality being the functional link of their antifungal activity (Shuster, *et al*, 1984) and (2) improvement in D/SD is almost invariably accompanied by reduction in scalp *Malassezia* level (Piérard *et al*, 1997; Gupta *et al*, 2004). Whereas the individual subject's abundance of scalp *Malassezia* cells does not to correlate with the presence or severity of D/SD, their reduction, among symptomatic individuals, strongly supports its role.

Also, the study of *Malassezia* has been complicated by their fastidious cultivation requirements and a complex series of changes in their nomenclature (Gueho *et al*, 2001; Gupta *et al*, 2001; Theelen *et al*, 2001; Gupta *et al*, 2004). *Malassezia* were initially identified and linked to dandruff and seborrheic dermatitis (D/SD) by a French scientist, Malassez, in the late 19th century (Malassez, 1874). In the 1950s they were reclassified into two species: the lipid-dependent *P. ovale* and the non-lipid-dependent *P. pachydermatis* (so named because it is found primarily on animals and not humans) (Salkin and Gordon, 1977). In the 1990s, it was determined that there were multiple species of the genus *Malassezia* (Guillot and Gueho, 1995), which now consists of ten lipid-dependent species: *globosa, restricta, furfur, slooffiae, sympodialis, japonica, nana, dermatis, yamatoensis* (Sugita *et al*, 2002; Sugita *et al*, 2003), and the non-lipid-dependent *pachydermatis*. We previously identified the *Malassezia* species correlating to D/SD as *M. restricta* and *M. globosa* (Gemmer *et al*, 2002), and proposed that dandruff is mediated by *Malassezia* metabolites, specifically irritating free fatty acids released from sebaceous triglycerides.

In this report, we report evidence of an underlying permeability barrier deficiency in individuals pre-disposed to D/SD. Present in human sebum (Wheatley, 1986), oleic acid (OA) was used as a representative *Malassezia*-produced, un-

Abbreviations: ASFS, Adherent Scalp Flaking Score; D/SD, dandruff and seborrheic dermatitis; OA, oleic acid; SG, sebaceous gland

saturated sebum metabolite to characterize scalp skin response to this class of lipids. The effect of any residual *Malassezia* was minimized by reducing their numbers by pretreating all subjects with a commercial pyrithione zinc-containing shampoo. Further, in order to preclude inadvertently increasing the number of *Malassezia*, the OA (a known *Malassezia* carbon source as well as metabolite) was dosed in an anti-microbial vehicle containing 50% propylene glycol (Faergemann and Fredriksson 1980; Faergemann, 2000). Propylene glycol has previously been used as a carrier for fatty acids used in human skin barrier studies (Tanojo *et al*, 1998).

Our results indicate that OA, administered to the human scalp, can induce flaking in dandruff-susceptible, but not non-susceptible individuals. This underlying skin barrier defect is a likely explanation for the lack of a numerical correlation between *Malassezia* numbers and D/SD. These data lend further support for a causal role of *Malassezia* in D/SD in that a sebum metabolite known to be produced by this organism is capable by itself of producing a lesion strikingly similar to ordinary dandruff. Moreover, these data may further explain how otherwise harmless, commensal microorganisms can cause skin damage.

Results

OA induces dandruff-like desquamation in dandruff subjects As shown in Fig 1, application of 7.5% OA induced flaking in subjects who had been previously identified as having dandruff. The two doses were statistically significant ($p < 0.1$) at days 5, 7, and 8. The effect was not related to growth induction of *Malassezia*, as their population was monitored throughout the study and there was no significant population change (data not shown).

OA, at the same dose, does not induce flaking in non-dandruff subjects As shown in Fig 2, subjects pre-determined to not suffer from dandruff do not have increased flaking when challenged with an equivalent dose of OA.

OA-induced flaking is ultrastructurally identical to normal dandruff In the subjects with OA-induced dandruff, the induced flaking was ultrastructurally identical to flakes isolated from normal dandruff. Previously, we reported the ultrastructure of D/SD *versus* normal scalp (Warner *et al*, 2001), and developed a scoring scale for the abnormal D/SD physiology. Comparison of D/SD with OA-induced flaking revealed an identical ultrastructure.

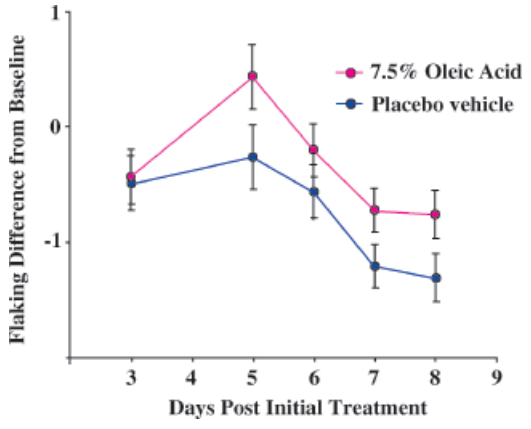

Figure 1
Dandruff-like flaking induced by oleic acid in subjects previously identified as dandruff susceptible. Flaking was significantly increased on days 5, 7, and 8, in the absence of *Malassezia*.

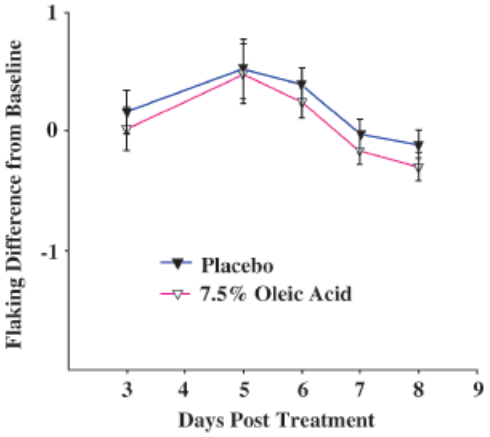

Figure 2
Dandruff-like flaking induced by oleic acid in subjects previously identified as not dandruff susceptible. Flaking was not significantly increased by application of oleic acid.

Discussion

Recent advances in the field of medical mycology, particularly with genetic-based detection methods, have increased interest and research on the causes of multiple skin disorders in which fungi play a role (Gupta *et al*, 2003, 2004). Also, genetically based species definition of the *Malassezia* genus has enabled detailed investigation of the appropriate species (Gaitanis *et al*, 2001; Gemmer *et al*, 2002). In an earlier report, we identified the *Malassezia* species commonly found on human scalp, and are now investigating the metabolism of *M. globosa and M. restricta in vitro* and *in situ*.

M. globosa was shown to have lipase activity, which hydrolyzed human sebum triglycerides into free fatty acids. Reduction of *Malassezia* populations has also been shown to correlate with D/SD reduction, but with no apparent correlation of absolute *Malassezia* numbers between D/SD individuals prior to treatment and non-D/SD individuals (Shuster, 1984).

In this report, we show that a representative *Malassezia* fatty acid metabolite (OA) is able to induce scalp flaking in susceptible individuals, but not in non-susceptible individuals. The vehicle chosen for dosing of OA (propylene glycol 50%, ethanol 30%, and water 20%) was selected to minimize the potential for increasing *Malassezia* numbers (Faergemann, 2000). The lack of response of non-susceptible individuals is particularly noteworthy, in that another report (Tanojo *et al*, 1998) showed significant barrier disruption effects from a slightly higher dose of OA in a similar vehicle. Our result strongly supports that dandruff sufferers display an underlying difference in permeability barrier function, relative to non-dandruff individuals, that renders them more susceptible to fatty acid-induced barrier disruption.

In this regard, dandruff susceptibility may be determined, at least in part, by a defect in basal permeability barrier function as is well established in the case of atopic dermatitis (Melnik *et al*, 1988; Imokawa *et al*, 1991; Leung and Bieber, 2003). These data also provide an explanation for the lack of a simple quantitative relationship between *Malassezia* species and D/SD presence or severity.

Integrating all available data, it appears that dandruff and seborrheic dermatitis most likely result from three specific etiologic factors: 1—Individual susceptibility; 2—Sebaceous secretion; and 3—*Malassezia* fungi. Individual susceptibility is most likely related to basal permeability barrier function, immune system function, and possibly even action of the overall microbial community.

Materials and Methods

This was a single-center study of 20 non-dandruff subjects (adherent scalp flaking scores (Van Abbe, 1964) (ASFS of ≤ 8) and 20 dandruff sufferers (ASFS of ≥ 24). Briefly, ASFS is the sum of a 0–10 scale for eight defined areas encompassing the entire scalp, yielding a 0–80 possible flaking scale. An ASFS of 15 represents flaking visible from 5 ft away. All subjects used a non-dandruff, non-conditioning shampoo for 2 wk prior to screening. Subjects refrained from shampooing for 72 h prior to screening, where their scalps were examined for dandruff severity. The two highest and two lowest sites were labeled with a Sharpie (Sanford Corp., Oakbrook, Ill.). The marks were reapplied at each visit to prevent wash-off.

All qualifying subjects were treated with 1% Pyrithione-Zinc (PtZ)-containing shampoo for 3 wk prior to baseline, to lower their ASFS and reduce *Malassezia* to background. All subjects remained on the 1% PtZ-containing shampoo throughout the study (subjects were monitored for *Malassezia* load, and no significant differences were seen due to experimental treatment (data not shown)).

At baseline, subjects were graded for dandruff severity, shampooed on site, and high and low flaking sites were dosed with active and placebo. Each subject received both placebo (propylene glycol 50%, ethanol 30%, water 20%) and active (7.5% OA in placebo) on high and low flaking sites (high $\pm$ OA, low $\pm$ OA).

At each subsequent visit (days 3, 5, 6–8), the subjects scalps were graded for dandruff severity, shampooed on site, and then dosed (active or placebo applied to the same sites). On day 8, the scalp was graded for dandruff severity, high and low sites were swabbed for microbiology, and stratum corneum specimens were obtained via tape stripping. Tape strips were examined by described techniques to highlight the lipid structure (Warner *et al*, 1999, 2001).

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