

## A New Calcineurin Inhibitor, Pimecrolimus, Inhibits the Growth of *Malassezia* spp.

*Malassezia* spp. are a component of the cutaneous microflora that colonizes lipid-rich areas, especially the head and neck, because the microorganisms require a lipid for its growth. An anti-*Malassezia*-specific immunoglobulin E antibody is produced in patients with atopic dermatitis who have disrupted skin barrier function, while healthy subjects do not produce the immunoglobulin E antibody. In addition, antifungal agents can improve the symptoms of atopic dermatitis. Based on this evidence, *Malassezia* spp. are considered one of the factors involved in exacerbating atopic dermatitis (1, 4). The genus *Malassezia* consists of 11 species (*M. dermatis*, *M. globosa*, *M. furfur*, *M. japonica*, *M. obtusa*, *M. restricta*, *M. slooffiae*, *M. sympodialis*, *M. yamatoensis*, *M. nana*, and *M. pachydermatis*), although the last two species show affinities to nonhuman animals. Of the 11 species, *M. globosa* and *M. restricta* have been isolated from almost all patients with atopic dermatitis, while the other species are found in fewer than 60% of patients, suggesting that *M. globosa* and *M. restricta* play a major role in atopic dermatitis (5).

Pimecrolimus, an ascomycin macrolactam derivative, is a new calcineurin inhibitor that binds to the cytosolic receptor macrophilin-12 with high affinity, inhibiting the calcium-dependent phosphatase calcineurin, an enzyme required for the dephosphorylation of the cytosolic form of the nuclear factor of the activated T cell. Therefore, it targets T-cell activation and proliferation by blocking the release of both TH1 and TH2 cytokines (7). Previously, we demonstrated that the calcineurin inhibitor tacrolimus, which has a similar chemical structure, inhibits the growth of *Malassezia* in vitro (6). Therefore, we postulated that pimecrolimus might have an antifungal effect.

In this study, we examined the in vitro drug susceptibility to pimecrolimus of 109 strains of the nine human-related *Malassezia* species. The strains were isolated from patients with atopic dermatitis or from healthy subjects. Pimecrolimus was kindly supplied by Novartis (Basel, Switzerland). In vitro drug susceptibility was determined according to the method of Gupta et al. (3), with slight modification (6). Drug susceptibility testing was conducted at least three times.

The MICs of pimecrolimus are shown in Table 1. Pimecrolimus had an antifungal effect against the 109 *Malassezia* strains, with MICs ranging from 16 to 64  $\mu\text{g/ml}$ . It inhibited the growth of approximately 90% of the strains at a concentration of 16 or 32  $\mu\text{g/ml}$ . No differences in MICs were seen across the *Malassezia* species. The calcineurin inhibitor tacrolimus, which has a similar chemical structure, also inhibited the growth of *Malassezia* in vitro, with MICs of 16 to 32  $\mu\text{g/ml}$ , which were the same as the MICs of

TABLE 1. Antifungal susceptibilities of *Malassezia* strains to pimecrolimus

| Species               | No. of strains | Cumulative % inhibited at the following MIC <sup>a</sup> ( $\mu\text{g/ml}$ ) |           |          |
|-----------------------|----------------|-------------------------------------------------------------------------------|-----------|----------|
|                       |                | 16                                                                            | 32        | 64       |
| <i>M. globosa</i>     | 25             | 48.0 (12)                                                                     | 48.0 (12) | 4.0 (1)  |
| <i>M. restricta</i>   | 25             | 40.0 (10)                                                                     | 44.0 (11) | 16.0 (4) |
| <i>M. sympodialis</i> | 20             | 80.0 (16)                                                                     | 20.0 (4)  |          |
| <i>M. slooffiae</i>   | 11             | 54.5 (6)                                                                      | 36.4 (4)  | 14.3 (1) |
| <i>M. furfur</i>      | 9              | 22.2 (2)                                                                      | 66.7 (6)  | 11.1 (1) |
| <i>M. obtusa</i>      | 12             | 8.3 (1)                                                                       | 41.7 (5)  | 50.0 (6) |
| <i>M. dermatis</i>    | 3              | 100 (3)                                                                       |           |          |
| <i>M. japonica</i>    | 2              | 50.0 (1)                                                                      | 50.0 (1)  |          |
| <i>M. yamatoensis</i> | 2              | 50.0 (1)                                                                      | 50.0 (1)  |          |

<sup>a</sup> The number of strains examined is shown in parentheses.

pimecrolimus (6). Interestingly, fungal cells also contain a calcineurin homologue, although its function is unknown. Recent studies indicate that cyclosporine and tacrolimus are toxic to the pathogenic fungi *Candida albicans* and *Cryptococcus neoformans* (2).

*Malassezia* spp. are one of the factors that exacerbate atopic dermatitis. The growth-inhibitory effect of pimecrolimus might contribute to improving the symptoms of atopic dermatitis in addition to the inhibitory effect of calcineurin as its main action. A 1% concentration of pimecrolimus is used for clinical purposes, which sufficiently exceeds the growth-inhibitory concentration for *Malassezia*.

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