Antifungal Activities of the Combination of Tacrolimus and Itraconazole Against Trichophyton mentagrophytes

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(Received 26 October 2004/Accepted 1 March 2005)

ABSTRACT. Tacrolimus was reported to have no antifungal activity against dermatophytes, but it might show a synergistic fungicidal activity with azoles against dermatophytes as in the case of pathogenic yeasts. Therefore, we examined the antifungal activity of tacrolimus combined with itraconazole (ITZ) against five strains of Trichophyton mentagrophytes by measuring cell growth and performing MIC (minimum inhibitory concentration) testing. The mean colony diameter of T. mentagrophytes on 1/10 diluted Sabouraud dextrose agar (dSDA) with tacrolimus combined with ITZ was significantly shorter than that on dSDA with tacrolimus or ITZ. MICs of tacrolimus, ITZ, and tacrolimus combined with ITZ against T. mentagrophytes were determined, respectively. The results revealed a synergistic activity of tacrolimus in combination with ITZ against T. mentagrophytes.

KEY WORDS: antifungal activities, tacrolimus, Trichophyton mentagrophytes.

Immunosuppressive drugs such as tacrolimus (FK506), sirolimus (rapamycin), and cyclosporin A (CsA) bind to the immunophilin, a member of prolyl isomerases family. Tacrolimus and sirolimus bind to the FKBP51-binding proteins, while CsA binds to cyclophilins. FKBP51s highly preserved in prokaryotic and eukaryotic cells, are found in multiple intracellular compartments, and are related to the suppression of the T-cell functions. The FK506-FKBP51 complex blocks T-cell activation [3, 4], and inhibits the protein phosphatase calcineurin.

Tacrolimus was suggested to have the antifungal activity, and to show the synergistic fungicidal or fungistatic activity with azoles against pathogenic fungi such as Cryptococcus neoformans, Candida albicans and Malassezia furfur. Additionally, it was reported that the synergistic activity was related to the FK506-FKBP51 complex [1, 2, 7].

Tacrolimus was reported to have no antifungal activity against dermatophytes isolated from humans and animals [7], but the synergistic fungicidal activity with azoles against dermatophytes has not been examined adequately. In order to elucidate the metabolic mechanism of the antifungal drug in dermatophytes, the antifungal activity of tacrolimus combined with the triazole antifungal drug, itraconazole (ITZ) against five strains of Trichophyton mentagrophytes (Arthroderma benhamiae) (Table 1) were examined.

Firstly, the effect on fungal cell growth of tacrolimus (Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan) and ITZ (Janssen Pharmaceutical Co., Ltd., Beerse, Belgium) was examined, according to the report of Kano et al. [5]. A fungal suspension in physiological saline was filtered through a membrane filter (40 μm) to obtain the fungal inoculum, which mostly consisted of microconidia, and the cells in the filtered suspension were enumerated by hemocytometer.

Approximately 1,000-cells/2 μl of each strain were inoculated in a 3 mm diameter spot onto the plates of 1/10 diluted Sabouraud dextrose agar (dSDA) [6] and dSDA with tacrolimus (0.005 μg/ml) and/or ITZ (0.001 μg/ml). Each of the strains was cultured for 1 week at 27°C, the diameters of the fungal colonies were then calculated, and these values were analyzed using the paired, two-tailed Student’s t-test. These procedures were carried out three times. All diameters are shown as the means ± standard deviation (SD). The results for the mean colony diameter of dSDA with these two drugs was significantly shorter than that of the other media [3.00 ± 0.1 cm for dSDA, 2.2 ± 0.7 cm for dSDA with tacrolimus, 1.9 ± 0.3 cm for dSDA with ITZ, and 1.4 ± 0.4 cm for dSDA with tacrolimus and ITZ] (Fig. 1).

Secondly, the in vitro synergistic activities of tacrolimus and itraconazole against T. mentagrophytes was examined by the Checkerboard broth microdilution method [2], according to the recommendations of the National Commit-

NOTE Internal Medicine

**Table 1.** Strains of T. mentagrophytes used in this study

<table>
<thead>
<tr>
<th>Strains</th>
<th>Mating type</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>VUT-77011</td>
<td>+</td>
<td>IAM 12704=RV 26678</td>
</tr>
<tr>
<td>VUT-97010</td>
<td>–</td>
<td>Rabbit</td>
</tr>
<tr>
<td>VUT-00001</td>
<td>–</td>
<td>Guinea Pig</td>
</tr>
<tr>
<td>VUT-00002</td>
<td>–</td>
<td>Rabbit</td>
</tr>
<tr>
<td>VUT-00003</td>
<td>–</td>
<td>Rabbit</td>
</tr>
</tbody>
</table>

IAM: Institute of Applied Microbiology, University of Tokyo, Tokyo, Japan. RV: Institute de Medecine Tropicale, Antwerp, Belgium. VUT: School of Veterinary Medicine, University of Tokyo, Tokyo, Japan.
H. OZAWA ET AL.

 tee for Clinical Laboratory Standards (NCCLS) [8]. In regard to the method in this study, final concentrations were 0.781–100 \( \mu g/ml \) for tacrolimus, and 0.016–16 \( \mu g/ml \) for ITZ. Subsequently, the fungal cells were incubated at 27\( ^\circ \)C. The MIC endpoints were determined based upon score 2 (prominent reduction in growth or approximately 50% of the growth control) according to NCCLS [8].

Drug interactions were classified as synergistic, additive, autonomous, or antagonistic on the basis of the fractional inhibitory concentration (FIC) index [2]. The FIC indexes were calculated as follows. The interaction was defined as synergistic when the FIC index was less than 1.0, as additive when the FIC index was 1.0, as autonomous when the FIC index was between 1.0 and 2.0, and as antagonistic when the FIC index was more than 2.0 [2].

The MICs based on the checkerboard broth microdilution method and FIC indexes are shown in Table 2.

In this study, a strain-dependent difference was shown in the antifungal activity of tacrolimus, and the synergistic activity of tacrolimus in the combination with ITZ against \( T. \) mentagrophytes was demonstrated. However, the mechanism of the synergistic activity in dermatophytes is now under the examination.

Tacrolimus has been reported to enhance antifungal activity of azoles against \( C. \) neoformans and \( C. \) albicans [1, 2], and the antifungal mechanisms of tacrolimus combined with azoles against pathogenic fungi have been proposed. Del Poeta et al. reported that the combination of tacrolimus and fluconazole had a synergistic activity against \( C. \) neoformans. The synergistic activity was independent of FKBP12 and calcineurin, and might involve the ability of tacrolimus to inhibit multidrug resistance pumps, which are known to export azoles from fungal cells of \( C. \) neoformans [2]. However, Cruz et al. reported that calcineurin and FKBP12 mediated the effects of tacrolimus with azoles against \( C. \) albicans, and that there was no need to invoke multidrug resistance pumps [1]. On the other hand, calcineurin is required for some yeasts to survive under cell membrane stress in response to azoles exposure [1].

Since the synergistic activity of tacrolimus and ITZ against dermatophytes might depend on the above-mentioned mechanisms, the molecular mechanism of FKBP1s and calcineurin on the synergistic activity of tacrolimus and azoles against dermatophytes should be clarified.

REFERENCES


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**Table 2.** \textit{In vitro} synergistic activities of tacrolimus and itraconazole against \( T. \) mentagrophytes

<table>
<thead>
<tr>
<th>Strain</th>
<th>Tacrolimus (µg/ml)</th>
<th>ITZ (µg/ml)</th>
<th>Combined FIC index</th>
</tr>
</thead>
<tbody>
<tr>
<td>VUT-77011</td>
<td>&gt;100</td>
<td>0.5</td>
<td>25/0.031</td>
</tr>
<tr>
<td>VUT-97010</td>
<td>0.781</td>
<td>0.25</td>
<td>0.390/0.125</td>
</tr>
<tr>
<td>VUT-00001</td>
<td>6.25</td>
<td>0.5</td>
<td>0.781/0.125</td>
</tr>
<tr>
<td>VUT-00002</td>
<td>25</td>
<td>2</td>
<td>6.250/0.125</td>
</tr>
<tr>
<td>VUT-00003</td>
<td>12.5</td>
<td>0.063</td>
<td>6.250/0.016</td>
</tr>
</tbody>
</table>

a) Minimum inhibitory concentration.

b) Itraconazole.

c) Fractional inhibitory concentration.