Comparison of the In Vitro Activities of Newer Triazoles and Established Antifungal Agents against *Trichophyton rubrum*

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One hundred eleven clinical *Trichophyton rubrum* isolates were tested against 7 antifungal agents. The geometric mean MICs of all isolates were, in increasing order: terbinafine, 0.03 mg/liter; voriconazole, 0.05 mg/liter; posaconazole, 0.11 mg/liter; isavuconazole, 0.13 mg/liter; itraconazole, 0.26 mg/liter; griseofulvin, 1.65 mg/liter; and fluconazole, 2.12 mg/liter.

TABLE 1 Geometric mean MICs, MIC ranges, MIC50s, and MIC90s obtained by susceptibility testing of antifungal agents against 111 *T. rubrum* clinical isolates

<table>
<thead>
<tr>
<th>Drug</th>
<th>MIC/MEC (mg/liter)</th>
<th>Range</th>
<th>50%</th>
<th>90%</th>
<th>Geometric mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Griseofulvin</td>
<td>1–4</td>
<td>2</td>
<td>2</td>
<td>1.65</td>
<td></td>
</tr>
<tr>
<td>Fluconazole</td>
<td>0.125–64</td>
<td>2</td>
<td>64</td>
<td>2.12</td>
<td></td>
</tr>
<tr>
<td>Itraconazole</td>
<td>0.031–16</td>
<td>0.5</td>
<td>2</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>Voriconazole</td>
<td>0.031–16</td>
<td>0.031</td>
<td>0.125</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Posaconazole</td>
<td>0.016–1</td>
<td>0.125</td>
<td>0.5</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>Isavuconazole</td>
<td>0.031–4</td>
<td>0.06</td>
<td>0.125</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>Terbinafine</td>
<td>0.008–0.06</td>
<td>0.031</td>
<td>0.06</td>
<td>0.03</td>
<td></td>
</tr>
</tbody>
</table>

Dermatophytosis caused by *Trichophyton rubrum* is the most common cutaneous fungal infection worldwide (1), which represents the cause of between 80% and 90% of all chronic and recurrent infections (2). These infections establish an important public health problem because of the prolonged treatment required for the disease, because of the frequent recurrence of infection, and because they are generally considered difficult to manage (3). Reliable *in vitro* susceptibility testing would therefore be useful for selecting the most suitable antifungal treatment. For many years, griseofulvin was the only approved systemic antidermatophytic agent (4). However, nowadays, many potent antifungal agents are available for the treatment of dermatophytosis, such as allylamines and triazoles, which have more potent activity and fewer side effects (5–19). The expansion of information on *in vitro* susceptibility testing of dermatophytes to new antifungal agents will help in the selection and development of antifungal drug regimens.

The aim of the current study was to compare *in vitro* the activities of three newer triazoles, voriconazole, posaconazole, and isavuconazole, and four established antifungal agents against *T. rubrum* infection. One hundred eleven clinical isolates of *T. rubrum* were collected from seven dermatology clinics in Shanghai, China. Morphological identifications were confirmed by sequence-based analysis of the internal transcribed spacer of the rRNA gene region. The *in vitro* activities of seven antifungal agents were determined according to the CLSI reference guideline M38-A2 (20), with minor modifications. Two reference strains, *Trichophyton mentagrophytes* (strain ATCC MYA-4439) and *Candida parapsilosis* (strain ATCC 22019), were included as quality controls. Student’s *t* test with the statistical SPSS package (version 9.0) was used, and *P* values of <0.05 were considered statistically significant.

Table 1 lists the MIC ranges, geometric mean (GM) MIC50s, and MIC90s of seven antifungal agents against *T. rubrum* strains. Terbinafine, voriconazole, posaconazole, isavuconazole, itraconazole, and griseofulvin had low MICs against all tested strains, whereas fluconazole did not show inhibitory effects. Similar results have been achieved in other studies (Table 2); however, limited data are available for the newer triazoles isavuconazole and posaconazole.

Terbinafine was one of the most effective antifungal agents against *T. rubrum* among the 7 fungal agents tested, and our findings confirm those of previous studies (5–19) (Table 2).

We compared the *in vitro* activities of the 3 newer triazoles isavuconazole, posaconazole, and voriconazole with that of itraconazole. Three newer triazoles offered good *in vitro* activity against *T. rubrum* (Table 1). All isolates were far more susceptible to the 3 newer triazoles than to itraconazole (Table 1) and comparable to those reported by other studies (7, 9, 10, 14, 17, 18).

Isavuconazole is a novel broad-spectrum triazole agent and has the same mechanism of action as the other triazoles. Several studies have supported its efficacy in invasive *Candida* species, *Cryptococcus neoformans*, *Aspergillus* species, and *Mucorales* isolates.
TABLE 2 | Table 2: Summary of in vitro susceptibility of T. rubrum to antifungal drugs in different studies from 2000 to 2014

<table>
<thead>
<tr>
<th>Antifungal</th>
<th>Method</th>
<th>No. of incubation times</th>
<th>Incubation temperature</th>
<th>GM</th>
<th>Range</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terbinafine</td>
<td>M38-A</td>
<td>0.008 to 0.015</td>
<td>0.008 to 0.015</td>
<td>0.015</td>
<td>0.015 to 0.06</td>
<td>This paper</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>M38-A</td>
<td>0.015 to 0.06</td>
<td>0.015 to 0.06</td>
<td>0.06</td>
<td>0.06 to 2</td>
<td>This paper</td>
</tr>
<tr>
<td>Voriconazole</td>
<td>M38-A</td>
<td>0.06 to 2</td>
<td>0.06 to 2</td>
<td>2</td>
<td>2 to 8</td>
<td>This paper</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>M38-A</td>
<td>0.125 to 1</td>
<td>0.125 to 1</td>
<td>1</td>
<td>1 to 2</td>
<td>This paper</td>
</tr>
<tr>
<td>Posaconazole</td>
<td>M38-A</td>
<td>0.05 to 2</td>
<td>0.05 to 2</td>
<td>2</td>
<td>2 to 8</td>
<td>This paper</td>
</tr>
<tr>
<td>Griseofulvin</td>
<td>M38-A</td>
<td>0.11 to 0.5</td>
<td>0.11 to 0.5</td>
<td>0.5</td>
<td>0.5 to 2</td>
<td>This paper</td>
</tr>
</tbody>
</table>

Incubation time is in hours, unless otherwise stated.

PDA, potato dextrose agar; OA, oatmeal agar.

The geometric mean (GM) is the geometric average of the MIC values, which is more suitable for comparing the MICs of different antifungal agents. The MIC90 is the concentration at which 90% of the isolates are inhibited. The lowest GM was 0.05 mg/liter (15), followed by 0.125 mg/liter (9, 10), and 0.25 mg/liter (11, 12, 13, 14). However, the antifungal susceptibility profile of dermatophytes remains poorly examined. Ghannoum and Isham reported that isavuconazole had shown potent in vitro activity against dermatophytes (22) and was more active than other triazoles tested (itraconazole and voriconazole), but it had a higher MIC than that of terbinafine; however, against T. rubrum isolates with high MICs to terbinafine, the isavuconazole MICs remained low (0.06 mg/liter for all tested isolates) (23). In our study, the MICs of isavuconazole (GM, 0.13 mg/liter; MIC90, 0.125 mg/liter) were similar to those of posaconazole (GM, 0.11 mg/liter; MIC90, 0.5 mg/liter) and voriconazole (GM, 0.05 mg/liter; MIC90, 0.125 mg/liter); the difference was within 1 log2-dilution step, which was much lower than those of itraconazole (GM, 0.26 mg/liter; MIC90, 2 mg/liter) for the majority of the T. rubrum isolates tested.

Posaconazole showed activity similar to that described by Gupta, Kohli, and Batra (14), who reported posaconazole to be the most active compound, with an MIC of ≤0.5 mg/liter; the MIC90 was 0.5 mg/liter in our study. Similar data were reported by Singh, Zaman, and Gupta (10); however, the MIC was greater than that reported by us and Gupta, Kohli, and Batra (14). This variation may be a result of the different methods used (Table 2). The potent activity of posaconazole against Trichophyton violaceum (T. rubrum complex) has been reported by us as well (24).

The excellent activity of voriconazole against T. rubrum has been observed by B. Fernández-Torres et al. (17) and A. J. Carriilo-Muñoz et al. (9), with sample sets of 144 and 139 isolates, respectively (GM for both, 0.06 mg/liter). Our findings with 111 isolates have confirmed this good activity (GM, 0.05 mg/liter). There were, however, some discrepancies; in two of the previous reports, voriconazole appeared to be less active than itraconazole (7, 18). This could be attributed, at least partially, to the different methodology employed and the lack of standardized protocols. Our previous study (24) revealed that voriconazole had potent activity against T. violaceum.

For itraconazole, significant variations are shown in the published literature (Table 2). Overall, the geometric mean MIC of itraconazole for half of the isolates was <0.1 mg/liter, and the highest GM was 0.59 mg/liter (16), followed by 0.42 mg/liter (8). Our results showed good in vitro activity of itraconazole against T. rubrum (GM, 0.26 mg/liter); however, itraconazole was less active than the three new triazoles tested.

Griseofulvin was the first-line antifungal agent for the treatment of dermatophytoes for many years, but today, it is not widely used (4), due to griseofulvin-resistant isolates of dermatophytes and the existence of strains with elevated MICs to griseofulvin (6, 25–27). With our results, the MICs of griseofulvin for T. rubrum were in agreement with those reported by Adimi et al. (7) and Perea et al. (18). Griseofulvin was less active than the rest of the agents tested except for fluconazole against T. rubrum. Nevertheless, all strains were more susceptible to griseofulvin than to fluconazole (Table 1). Among the studies reported in Table 2, fluconazole also was effective against T. rubrum, except in a study by Adimi et al. (7). Of all the agents tested in the current study, fluconazole showed the lowest activity, which was consistent with previous studies (9, 10, 16); although T. rubrum is not susceptible to fluconazole, it is recommended for the management of some dermatophytoes (28–30).

In conclusion, terbinafine, voriconazole, posaconazole, and isavuconazole were shown in vitro to be the most potent antifun-
gal agents against the *T. rubrum* isolates investigated. These results might help clinicians to develop appropriate therapies for treating dermatophytosis caused by *T. rubrum*. However, further clinical investigations must be conducted in order to develop interpretive breakpoints.

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