In Vitro Activities of Azole Antifungal Agents against Propionibacterium acnes Isolated from Patients with Acne Vulgaris

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The Gram-positive bacterium Propionibacterium acnes is the causative agent of acne vulgaris. Antibiotics such as tetracycline and macrolide derivatives are used to treat this skin disease; however, the isolation frequency of antibiotic-resistant P. acnes has been increasing. The anti-P. acnes activity of imidazole antifungal agents was reported more than 20 years ago, and since then, new azole antifungal agents have been marketed. Thus, this study determined the in vitro activities of azole antifungal agents against P. acnes isolated from patients with acne vulgaris. Of the five agents tested, miconazole, ketoconazole, and itraconazole showed concentration-dependent anti-P. acnes activity, including against antibiotic-resistant isolates. Time-kill assay also showed the time-dependent activity of the drugs. Fluconazole and voriconazole showed no anti-P. acnes activity.

Keywords Propionibacterium acnes; acne vulgaris; azole agent; anti-bacterial activity; time-kill assay

Propionibacterium acnes is a Gram-positive anaerobic to aero-tolerant bacterium and part of the skin microbiota in humans. It colonizes areas of skin rich in sebaceous glands, such as the scalp and face. The amount of P. acnes in skin is related to the activity of the sebaceous glands; the population increases after the maturation of sebaceous gland function following puberty.1)

P. acnes is the major etiological agent of inflammatory acne, and multiple mechanisms are involved in this inflammatory process. P. acnes stimulates the release of interleukin-1 (IL-1), IL-8, and tumor necrosis factor-α (TNF-α) and activates the complement system. This microorganism also produces free fatty acids via the hydrolysis of sebaceous gland triglycerides by its lipase. A wide range of antibiotic classes are effective against acne vulgaris due to P. acnes, such as clindamycin, erythromycin, quinolones, and the tetracycline family. In the last decade, the appearance of antibiotic-resistant P. acnes has increased.2) A European surveillance study suggested that 15.1% of clinical isolates were resistant to clindamycin and 17.1% to erythromycin.3)

In the 1980s, a double-blind trial of a lotion containing 2% miconazole (MCZ) and 5% benzoyl peroxide was performed in patients with acne vulgaris.4) High efficacy was seen in 88% of the combination group compared to the group using benzoyl peroxide alone, with a significant reduction in inflammatory lesions. Although MCZ is an imidazole antifungal agent, the compound is also active against Gram-positive bacteria, such as P. acnes, Staphylococcus aureus, and Corynebacterium spp., in vitro.5,6) The compound causes the release of cellular K+6,7), which may be related to membrane damage.6,7) More than 20 years have passed since the clinical effectiveness of MCZ against acne vulgaris due to P. acnes was first elucidated. Since then, several azole agents have been introduced for the clinical treatment of fungal infections. In this study, we reassessed the in vitro activities of several azole antifungal agents against P. acnes.

MATERIALS AND METHODS

Materials Thirty-two clinical P. acnes isolates were obtained from patients with acne vulgaris. Of them, eight were resistant to both erythromycin [minimum inhibitory concentration (MIC), 8—16 µg/ml] and clindamycin (MIC, 32—64 µg/ml), while all were susceptible to penicillin G (MIC, <0.25 µg/ml), ampicillin (MIC, <0.25 µg/ml), piperaclillin (MIC, <8 µg/ml), cefoxitin (MIC, <4 µg/ml), ceftriaxone (MIC, <0.5 µg/ml), minocycline (MIC, <1 µg/ml), chloramphenicol (MIC, 4 µg/ml), and tosufloxacin (MIC, <0.5—1 µg/ml). MCZ, fluconazole (FLZ), itraconazole (ITZ), and voriconazole (VRZ) were purchased from ASTY (Kyokuto Pharmaceutical, Tokyo, Japan); ketoconazole (KTZ) was obtained from Jassen Pharmaceutical (Tokyo, Japan).

Drug Susceptibility Testing The in vitro activities of the five azole agents were determined at concentrations of 0.125—8 µg/ml in reduced Brucella broth (Japan Becton Dickinson, Tokyo, Japan) supplemented with 5 mg/l hemin (Sigma-Aldrich, Tokyo, Japan) and 1 mg/l vitamin K1 (Sigma-Aldrich) according to the method of Tyrrell et al.8) The plates were incubated in an anaerobic chamber at 35°C for 48 h. Aliquots of the cultures were taken from each well, diluted serially, and plated onto supplemented Brucella agar plates for counting of the colony-forming units (CFUs).

Time-Kill Assay A time-kill assay was performed using the same reduced Brucella broth as in our drug susceptibility tests. The drug concentrations used were 0, 0.5, 2, and 8 µg/ml. The drugs and strains were added to the tubes in an anaerobic chamber. The final inoculum was approximately 5×10^5 CFUs/ml. Aliquots were removed after 0, 6, 12, 24, and 48 h and plated onto supplemented Brucella agar plates to determine the number of CFUs.
RESULTS AND DISCUSSION

Table 1 shows the reduction rate (%) of CFUs for each antibiotic-susceptible and -resistant clinical isolate of *P. acnes* in agent-containing and -free solutions of MCZ, ITZ, and KTZ. Each agent reduced the number of CFUs of *P. acnes* in a concentration-dependent manner (0.5—8 μg/ml). At 8 μg/ml, the agents inhibited the growth of the microorganism by more than 80% compared to the control. Of the three compounds, MCZ showed the most potent activity against *P. acnes*, followed by KTZ and ITZ. This study included eight erythromycin- and clindamycin-resistant clinical isolates. No remarkable difference in drug susceptibility was seen between the antibiotic-susceptible and -resistant isolates. FLZ and VRZ did not reduce the CFUs of any strain at a concentration of 0.5—8 μg/ml. Time-kill assays for each representative

<table>
<thead>
<tr>
<th>Agent</th>
<th>Concentration (μg/ml)</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Miconazole</td>
<td></td>
<td>51.9±13.1 (a)</td>
<td>74.5±15.1</td>
<td>83.6±10.5</td>
<td>91.9±5.6</td>
<td>94.0±4.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>62.9±13.3 (c)</td>
<td>79.5±12.5</td>
<td>88.0±4.9</td>
<td>95.1±4.5</td>
<td>96.8±2.2</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td></td>
<td>28.9±11.2</td>
<td>51.8±10.5</td>
<td>59.9±9.1</td>
<td>75.1±9.1</td>
<td>89.5±5.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30.2±11.3</td>
<td>52.1±11.3</td>
<td>60.1±18.9</td>
<td>76.0±10.9</td>
<td>88.5±7.2</td>
</tr>
<tr>
<td>Itraconazole</td>
<td></td>
<td>20.2±8.4</td>
<td>34.3±8.2</td>
<td>46.9±7.3</td>
<td>72.6±2.8</td>
<td>86.1±4.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>19.2±9.0</td>
<td>33.9±7.8</td>
<td>44.0±6.0</td>
<td>71.7±3.1</td>
<td>84.9±4.9</td>
</tr>
</tbody>
</table>

The percent reduction was calculated as the number of CFUs in the agent-containing solution relative to that in the agent-free solution. *a*) Antibiotic-susceptible strains (n=24). *b*) Antibiotic-resistant strains (n=8). *c*) Mean±standard deviation.

![Time-Kill Assays](image-url)
antibiotic-susceptible and -resistant strain were carried out using MCZ, KTZ, and ITZ (Fig. 1). Although the drug concentration used in the assays was generally based on the MIC (e.g., 4—8×MIC), the assays were performed using 0, 0.5, 2, and 8 μg/ml each drug since no MIC was determined. The anti-\textit{P. acnes} activity of all of the drugs was time-dependent and 80% of the microorganisms were eliminated by all of the drugs after 6 h.

Azole agents exert their antifungal effects by inhibiting fungal ergosterol biosynthesis. \textit{Candida} and \textit{Aspergillus} are major causes of fungal infection. All azole agents are susceptible to \textit{Candida}, whereas variation in susceptibility to \textit{Aspergillus} has been reported. Structurally, MCZ and KTZ are “imidazole” agents, whereas ITZ, FLZ, and VRZ are “triazole” agents. Unfortunately, no relationship between the chemical structure and anti-\textit{P. acnes} activity of these agents has been found. Any evidence of such a relationship may contribute to the development of new anti-\textit{P. acnes} agents since the appearance of antibiotic-resistant \textit{P. acnes} is on the rise.

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**REFERENCES**