Evaluation of combined deactivators-supplemented agar medium (CDSAM) for recovery of dermatophytes from patients with tinea pedis

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Using a newly-developed medium, i.e., combined deactivators-supplemented agar medium (CDSAM), the viability of dermatophytes in skin scales was evaluated. Culture studies were conducted with skin scales collected from 44 patients with tinea pedis who had been treated for two weeks with topical antifungal drugs. Sixty-four percent of the specimens were mycologically positive on CDSAM, while only 23% of these same specimens were positive when cultured on conventional Sabouraud’s dextrose agar medium (SDA). Alternatively, 36% of all cases were negative on both media. The experimental data from this clinical study indicate that CDSAM was more useful than SDA in accurately evaluating the efficacy of antifungal drugs since the former minimized the residual effects of drug residues remaining in the skin samples.

Keywords medium, antifungal agents, tinea pedis, clinical study

Introduction

Several antifungal drugs, such as derivatives of imidazole/allylamine/benzylamine, morpholine, and thiocarbamate are currently in use for the topical treatment of dermatophytoses. These antifungals have the common characteristic of remaining active within the horny layer of the epidermis for a protracted periods of time [1,2]. In a clinical setting, mycological evaluation of antymycotics is usually carried out by examining fungal elements in skin scales using only the KOH direct-microscopic method. To determine the therapeutic benefits of antifungal drugs, portions of skin samples are inoculated onto Sabouraud’s dextrose agar (SDA). However, SDA is far from the best medium to support the growth of dermatophytes because of the carryover effect of drug residues within the specimens which can contribute to an overestimation of the efficacy of the antifungals. To overcome this problem, a new agar medium was devised which contained two surface-active deactivator compounds, i.e., egg lecithin and polysorbate 80, as described by Nakashima et al. in a guinea pig model of tinea pedis [3]. Previously these two surface-active compounds had been conventionally utilized as deactivators of various preservatives in food and cosmetics [4]. In this paper we have evaluated the efficacy of combined deactivators-supplemented agar medium (CDSAM) for the recovery of dermatophytes from skin scales collected from tinea pedis lesions in patients receiving topical antifungal therapy.

Patients and methods

Clinical assessment was carried out for tinea pedis patients whose initial diagnosis was made through KOH direct-microscopic examination and subsequently confirmed by culture. Patients were directed to apply an antifungal cream once daily for 4 weeks. After two weeks of treatment with bifonazole cream (Mycospor, Bayer Yakuhin, Ltd.), terbinafine (Lamisil, Novartis Pharma), lanoconazole (Astat, Tsumura & Co.), or butenafine (Mentax, KAKEN Pharmaceutical Co., Ltd.), skin scales were removed from an infected area and examined for fungal elements by KOH direct-microscopic procedures.

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Subsequently, other portions of the scales from these same patients were cut into equal-sized pieces and inoculated onto two different agar media, SDA and CDSAM [1,2]. The SDA contained per ml 500 μg of cycloheximide (Wako Pure Chemical Industries, Ltd), 50 μg of sisomicin (Sigma-Aldrich Co.), and 100 μg of chloramphenicol (Wako Pure Chemical Industries, Ltd), while CDSAM was the same medium to which was added 1% egg lecithin and 0.7% polysorbate 80.

Cultures yielding fungal growth were assessed as culture-positive after incubation at 30°C for 14 days. Statistical analysis was performed using McNemar’s Test.

Results

Forty-four patients with tinea pedis (interdigital:30, plantar:14) were entered in this study. The etiologic agents recovered were Trichophyton rubrum (25 patients) and T. mentagrophytes (19 patients) and in those instances in which the samples yielded growth on both media, identification of the isolate was found to be the same.

The persistence or eradication of the fungi in the skin was ascertained by culture after two weeks treatment. Cultures yielded T. rubrum on SDA with samples from 5 patients and on CDSAM with scales collected from 12 patients. In cases involving T. mentagrophytes, SDA cultures were positive with material from five patients but yielded the fungus on CDSAM with samples from 12 patients. There was no significant difference in growth between the two species. For the plantar type infections, culture positive assessments were obtained on SDA and CDSAM in 3 and 8 cases, respectively, while for the interdigital cases, cultures were positive for 7 and 20 cases, respectively (Table not shown).

Seventeen patients were treated with bifonazole and twenty-seven with other antifungal drugs (terbinafine (n=8), lanoconazole (n=7), and butenafine (n=12)). In patients receiving topical antifungotics samples from 10 cases were positive on SDA, while scales from 28 cases were positive on CDSAM. Of the total 44 patients in this study, specimens from 16 were culture negative and another 10 were culture positive on both media. In addition, samples from 18 patients were positive on CDSAM only, but no dermatophytes were recovered only on SDA. A significant statistical difference between the SDA positive rates and the CDSAM positive rates (SDA:10(22.7%) vs. CDSAM:28(63.6%)) was observed (Table 1, P<0.01, McNemar’s test). A comparison of culture positive rates by antifungal drug is summarized in Table 1.

<table>
<thead>
<tr>
<th>SDA</th>
<th>CDSAM</th>
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<tbody>
<tr>
<td>Positive</td>
<td>10</td>
</tr>
<tr>
<td>Negative</td>
<td>18</td>
</tr>
<tr>
<td>Total</td>
<td>44</td>
</tr>
</tbody>
</table>

Culture studies were performed after 2 weeks of treatment. Topical antifungotics: bifonazole (n=17), terbinafine (n=8), lanoconazole (n=7), and butenafine hydrochloride (n=12).

Discussion

Many antifungal drugs currently in use for therapy of dermatophytoses are retained within the horny layer of the epidermis for lengthy periods. In a clinical setting, it is desirable that the therapeutic effect against tinea pedis be confirmed through culture studies using skin scales from infected sites. However, when the drugs mentioned above, or others displaying similar biological characteristics are used in treatment, culture studies can be appreciably affected by drug residues contained in the specimen. In such cases, patients may be judged as cured as a result of false negative cultures. Therefore, when the treatment is curtailed and the concentration of antifungal drugs within the horny layer decreases over time, there may be a reactivation of the infection.

Several newly-developed antifungal drugs have demonstrated significantly beneficial effects in studies using experimental animal models of dermatophytosis, but the same results could not be obtained in a clinical setting [5]. One reason for this phenomenon is the inability to accurately determine the viability of fungi via the KOH direct-microscopic examination or by culture. In 2002, Nakashima et al. reported that CDSAM, a new medium containing egg lecithin and polysorbate 80, minimizes the carryover effect of the topical antifungal drugs [3]. The present study corroborates and extends these observations on the greater efficacy of CDSAM for mycological evaluation of the effect of antifungal drugs in tinea pedis.

Although several trial studies have recently been reported that evaluated fungal viability via neutral red staining [6,7] or with an RT-PCR method for mRNA [8], it is believed that CDSAM will be a more convenient and less expensive method for accurately determining the therapeutic effect of antifungal drugs.
References