Original Article

Dimethyl Sulfoxide (DMSO) Inhibits the Germination of Candida albicans and the Arthrospores of Trichophyton mentagrophytes

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Abstract

Dimethyl sulfoxide (DMSO) is commonly used as a solvent for antifungal drugs. Earlier the author has reported the inhibitory effect of DMSO on the growth of many strains of dermatophytes’ colonies in dermatel agar and proposed that this could cause the variations between results of different studies for the evaluation of the activities of antifungal drugs. In studies regarding the determination of the effect of antifungal drugs on the germination of arthrospores of dermatophytes it was observed that relatively higher concentrations of DMSO were being used as a solvent for the antifungal drugs, the final concentration in the media being 5%. Therefore, the present study was aimed at determining the effect of different concentrations of DMSO (1.25 to 10%) on the growth of germ tubes of arthrospores of Trichophyton mentagrophytes and Candida albicans, in glucose peptone broth. With DMSO 10% there was a negligible growth of germ tubes of both the arthrospores and yeast; between 2.5 and 7.5% there was a rather linear dose-related inhibitory effect; whereas 1.25% had insignificant effect from controls. The present study shows that besides other factors, variations in the results of the susceptibility tests of antifungal drugs might occur due to the effect of DMSO on the growth of fungi and differences in the final concentration of DMSO in the medium.

Key words: DMSO, Trichophyton mentagrophytes arthrospores, Candida albicans germ tubes, glucose peptone broth

Introduction

DMSO is a highly polar and stable substance with exceptional solvent property. It also acts as a penetrant of drugs through the skin. Five percent DMSO has also been added in fungal suspensions, as a cryoprotectant, for storage at very low temperature, −80 °C. DMSO 2% has been reported to significantly inhibit the growth of Candida species by broth dilution method, while 1% and below had insignificant effect. Recently, the author has reported the inhibitory effect of DMSO, even below 1%, on the growth of different strains of three important genera of dermatophytes (Trichophyton, Epidermophyton and Microsporum).

In published studies regarding the germination of arthrospores of dermatophytes it was observed that relatively higher concentrations of DMSO were being used as a solvent for the antifungal drugs, the final concentration in the growth media being up to 5% 5−7. Therefore, the present study was aimed at determining the effect of different concentrations of DMSO (1.25 to 10%) on the growth of germ tubes of arthrospores of different strains of a dermatophyte, Trichophyton mentagrophytes and a yeast, Candida albicans, in glucose peptone broth.

Materials and Methods

a. DMSO, glucose peptone agar & glucose peptone broth:

DMSO was obtained from SIGMA, USA. From 100% DMSO, 10, 7.5, 5, 2.5 and 1.25% dilutions were prepared in sterile distilled water.
Glucose peptone agar was obtained from OXOID, England and contained: glucose 2%, mycological peptone 1% and agar 1.45%. Forty-four and one half grams was suspended in 1 liter of distilled water and gently heated to dissolve completely. Then it was sterilized by autoclaving at 121 ºC for 15 minutes.

Glucose peptone broth was also obtained from OXOID, England containing: 4% glucose, 1% peptone broth. After mixing with an appropriate amount of distilled water, it was sterilized by autoclaving at 121 ºC for 15 minutes.

b. Arthrosposes of *Trichopyton mentagrophytes*

*Trichopyton mentagrophytes*( var *interdigitale*) arthrosposes were produced in glucose peptone agar, maintained at 37 ºC in the presence of 20% CO2 in air for two weeks. Surface growth was removed, suspended in phosphate buffered saline (PBS) and shaken on a vortex mixer. The suspension was filtered through glass wool to remove unbroken chains of arthrosposes. The filtered arthrosospore suspension was washed three times in PBS at 3000 g for 5 minutes and adjusted to a concentration of 1 x 10⁷/ml. The viability of arthrosposes was checked by using 0.3 ml of arthrosospore suspension in 0.9 ml glucose peptone broth incubated at 37 ºC for 12 hours on a rotary shaker at 100 rpm. An arthrosospore was considered to have germinated when a visible germ tube had developed, as seen in the microscope under high power (× 40).

The method was essentially the same as that of Hashimoto and Blumenthal, with some modifications 8).

c. Yeasts

*Candida albicans*, obtained from the clinical samples, was grown on glucose peptone agar. Surface growth was removed, suspended in PBS and shaken on a vortex mixer. The suspension was filtered through glass wool. The filtered yeast suspension was washed three times in PBS at 3000 g for 5 minutes and adjusted to a final concentration of 1 x 10⁷/ml. The viability of yeasts was checked by using 0.3 ml of yeast suspension in 0.9 ml glucose peptone broth incubated at 37 ºC for 3 hours on a rotary shaker at 100 rpm. A yeast was considered to have germinated when a visible germ tube had developed, as seen in the microscope under high power (× 40).

d. Anti-germination assay:

To 0.3 ml of the arthrosospore/yeast suspension was added 0.3 ml glucose peptone broth plus 0.6 ml of each dilution of DMSO mentioned above. For control purposes 0.3 ml of the arthrosospore suspension was added to 0.3 ml glucose peptone broth plus 0.6 ml of sterile distilled water to make a total of 1.2 ml, corresponding to the volume of test preparations. Incubation was carried out at 37 ºC on a rotary shaker (100 rpm) for 12 hours for arthrosposes and 3 hours for yeast. Three germination bottles were used for each concentration of DMSO.

e. Observations & statistical analysis:

At the end of the incubation period 100 microliters of glutaraldehyde (25%) was added to each germination bottle to stop the process of germination. From each germ bottle three mounts were made on glass slides and examined microscopically for germination (thus making nine observations for each dilution of DMSO). The % of germinating arthrosposes/yeasts was counted in each slide from a total of 100 cells observed in different fields of microscope. Thus a mean (with standard deviation) of nine observations was calculated for each concentration of DMSO tested.

The results of different concentrations of DMSO were compared with the controls by Student’s t-test. The study was conducted on three strains of *T. mentagrophytes* as well as three strains of *C. albicans* at different occasions.

Results

The effect of different concentrations of DMSO on the germination of arthrosposes of *T. mentagrophytes* and the growth of *C. albicans* germ tubes is given in Table 1 and 2, respectively. In both the species the growth of germ tubes was inversely related to the concentration of DMSO. With 10% DMSO there was almost complete inhibition of the growth. The growth gradually increased lowering concentration and the results of DMSO 7.5, 5 and 2.5% were significantly different from controls. However, with 1.25% DMSO the effect was insignificant.

Discussion

Poor agreement between different methods for the evaluation of anti-fungal drugs is commonly reported, especially for their activity against dermatophytes 9-11. Some of the possible factors causing this variability had also been investigated 11. Earlier, the author has proposed that besides other factors which could cause poor agreement among these methods, one important factor might be the effect of dimethyl suloxide (DMSO) on the growth of fungi, which was shown to inhibit the growth of many strains of dermatophytes in very low concentrations (less than 1%) 11.

Arthrosposes of dermatophytes remain dormant in
Table 1. % of germination of three strains of arthrospores of *T. mentagrophytes* in the presence of different concentrations of DMSO in glucose peptone broth (mean±SD, n=9)

<table>
<thead>
<tr>
<th>Arthrospores of <em>T. mentagrophytes</em></th>
<th>% DMSO in glucose peptone broth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Strain 1</td>
<td>4.11</td>
</tr>
<tr>
<td></td>
<td>± 1.45</td>
</tr>
<tr>
<td></td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Strain 2</td>
<td>4.56</td>
</tr>
<tr>
<td></td>
<td>± 1.81</td>
</tr>
<tr>
<td></td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Strain 3</td>
<td>2.67</td>
</tr>
<tr>
<td></td>
<td>± 1.03</td>
</tr>
<tr>
<td></td>
<td>&lt;0.001*</td>
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</tbody>
</table>

* P values when the results were compared with the controls by Student’s ‘t’ test.

Table 2. % of germ tubes of yeasts of *C. albicans* in the presence of different concentrations of DMSO in glucose peptone broth (mean±SD, n=9)

<table>
<thead>
<tr>
<th><em>Candida albicans</em></th>
<th>% DMSO in glucose peptone broth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Strain 1</td>
<td>0.89</td>
</tr>
<tr>
<td></td>
<td>± 0.93</td>
</tr>
<tr>
<td></td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Strain 2</td>
<td>0.67</td>
</tr>
<tr>
<td></td>
<td>± 1.21</td>
</tr>
<tr>
<td></td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Strain 3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>± 0.89</td>
</tr>
<tr>
<td></td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

*P values when the results were compared with the controls by Student’s ‘t’ test.

exfoliated skin for a long time [12, 13]. Their germination is an initial step in the pathogenesis of dermatophytosis [12, 13]. Drugs that could inhibit the germination of arthrospores would be useful for prophylaxis from dermatophytosis. Only a few studies have investigated antifungal drugs for anti-germination effects on arthrospores of dermatophytes [14, 15].

Many factors have been determined which could affect the germination of dermatophyte arthrospores, including: temperature, pH of the medium, oxygen, etc. [14, 15]. From the previous observations of the effect of DMSO on the filamentous growth of dermatophytes [16], it was thought that changes in the concentration of DMSO as a solvent for antifungal drugs might also affect the germination of their arthrospores and cause variations in the results. In the present study we found that 10% DMSO almost completely inhibited growth of germ tubes of both the arthrospores and yeast; between 2.5 and 7.5% there was a rather linear dose-related inhibitory effect, whereas the effect of 1.25% differed insignificantly from the controls.

The production of arthrospores was tried on many species of dermatophytes at different temperatures (25, 30, 37°C). However, *T. mentagrophytes* gave the maximum yield of arthrospores at 37°C and 20% CO2. In a few studies reported in the literature regarding the investigation of antifungal drugs for anti-germination effects arthrospores of only *T. mentagrophytes* have been used [14, 15].

In the antigermination assay, incubation was carried...
out at 37°C on a rotary shaker (100 rpm) for 12 hours for arthrospores and 3 hours for yeast. More than 90% germination of arthrospores has been reported in 7-8 hours. Similarly, in our initial experiments near 90% growth of germ tubes of the yeast was observed in 3-4 hours. Slowing the speed to below 50 rpm favoured clumping of yeast and arthrospores.

The lower concentrations of DMSO which had negligible effect on the germination of arthrospores and yeasts might have an additive/synergistic effect with the antifungal drugs, and this needs further investigation.

Acknowledgements

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References