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Original Article

The Effect of Dimethyl Sulfoxide (DMSO) on the Growth of Dermatophytes

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Abstract

Dimethyl sulfoxide (DMSO) is frequently used as a solvent for antifungal drugs in various studies to determine their MICs. Reports on comparative evaluation of methods for the susceptibility testing of antifungal drugs have shown there is poor agreement among methods. Besides other factors which could cause variability in the results, one important factor might be the effect of DMSO on the growth of fungi. The effect of DMSO on the growth of some species of *Candida* has been reported in the literature. The present study aimed at determination of the effect of different concentrations of DMSO (0.125 to 10%) on the growth of dermatophytes by agar diffusion method. There was no growth of fungi in 10% DMSO, between 1.25 and 5% there was a rather linear dose-related inhibitory effect on the growth, significantly different from the controls, and below 1% there was a variable effect among the species. DMSO down to 0.25% significantly inhibited the growth of most strains of *M. canis*. The lower concentrations of DMSO, which apparently do not affect the growth of fungi, may potentiate the effect of antifungal drugs.

Key words: DMSO, dermatophytes, dermasel agar, growth-diameter

Introduction

There is a rising incidence of serious fungal infections due to the use of anti-cancer and immunosuppressive drugs. Fungal infections also occur in chronic illnesses like diabetes mellitus and AIDS. Limitations in the efficacy and tolerability of the existing antifungal drugs have created an increased demand for the development of new antifungal drugs[1, 2] and reliable methods of *in vitro* testing of antifungal agents[3-5].

Many commercial companies like PASCO have developed their own methods for testing the susceptibility of antifungal drugs. In a comparative evaluation of the NCCLS (M27-A) broth dilution method, which is considered as a gold standard, and the PASCO (division of Becton-Dickinson, USA) broth dilution method, the overall agreements were 91% for fluconazole, 89% for amphotericin B and ketoconazole, 80% for flucytosine, 77% for terconazole, 66% for miconazole and only 53% for clotrimazole, when tested against 74 yeast isolates. The PASCO method also classified 9%, 3% and 4% fungi resistant against itraconazole, fluconazole and flucytosine, respectively, which were reported to be sensitive by the M27-A reference method[5]. Besides other factors, which could cause poor agreement among these methods for some of the drugs, one important factor might be the effect of dimethyl sulfoxide (DMSO) on the growth of fungi.

DMSO is a highly polar, stable substance with exceptional solvent property. It also acts as a penetrant of drugs through the skin, e.g. it has been shown to increase the effectiveness of idoxuridine in herpes simplex[6]. Five percent DMSO has also been added to fungal suspensions, as a cryoprotectant, for storage at very low temperature, −80℃[7].

The influence of various concentrations of DMSO (2, 1 and 0.5%) on the growth of

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Candida species has been investigated in one study, reporting that 2% DMSO significantly slowed the growth and lowered the growth curve in all 8 species of *Candida* tested, while 1%, and below had an insignificant effect on the kinetics of growth[4].

The present study was intended to investigate the effect of various concentrations of DMSO on the growth of different strains of three important genera of dermatophytes (*Trichophyton*, *Epidermophyton* and *Microsporum*), the fungi commonly causing skin, hair and nail infections.

Materials and Methods

a. DMSO & dermasel agar

DMSO was obtained from SIGMA, USA. The dermasel agar (containing: mycological peptone 1%, glucose 2%, agar 14.5%) was obtained from OXOID, England. 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°C for 10 minutes.

b. Isolation of dermatophytes

Twelve clinical isolates of three important genera of dermatophytes (*Trichophyton*, *Epidermophyton* and *Microsporum*) were used in the study, including one each for *Trichophyton mentagrophytes*, *Epidermophyton floccosum* and *Microsporum canis*; as well as another four strains of *T. rubrum* and five strains of *M. canis*. These strains were obtained from skin, hair or nail scrapings of patients with a clinical diagnosis of dermatophytosis. The specimens were initially inoculated to petri dishes containing Sabouraud dextrose agar with a supplement of chloramphenicol and cycloheximide. The dishes were then incubated at 30°C for 7–10 days, and the growth was identified by colonial morphology as well as by microscopy, after staining with lactophenol cotton blue.

c. The study design

The study was conducted in three parts. Firstly, three isolates, one from each dermatophyte genus: *T. mentagrophytes*, *E. floccosum* and *M. canis* were grown in dermasel agar containing 10, 5, 2.5, 1.25, 0.62, 0.31 and 0.15% DMSO, prepared by serial dilutions. Secondly, four strains of *T. rubrum* were grown in dermasel agar containing 2.5, 1.25, 0.62 and 0.31% DMSO. Thirdly, five strains of *M. canis* were grown in dermasel agar containing 4, 2, 1, 0.5, 0.25 and 0.125% DMSO. The selection of the concentrations of DMSO was based on the pilot study.

d. Antifungal susceptibility test for DMSO:

The isolates of various dermatophytes were sub-cultured on different sets of culture media, one set for each concentration of DMSO (mentioned above) and an extra set containing dermasel agar alone (as a control) for each strain of the dermatophytes. Four petri dishes were inoculated for each dilution of DMSO as well as for the controls.

A colonial disc, 5 mm in diameter, cut from the periphery of a 7–10 day old culture of dermatophytes in dermasel agar was aseptically inoculated onto different sets of media. The inoculated plates were incubated at 30°C. The cultures were examined on day 7 and 14, and results determined by measuring the mean diameter of dermatophyte growth[8].

From the observations on day 14 the mean and standard deviation of the diameter of fungal colonies was determined. Using Microsoft Excel, linear plots for the growth of dermatophytes in the presence of different concentrations of DMSO were made, placing the concentration of DMSO on the X-axis and the growth on the Y-axis.

e. Statistical analysis:

Mean values for the growth of dermatophytes at different concentrations of DMSO in dermasel agar were compared separately with the mean values of corresponding controls by the Student's t test. Statistically significant difference was considered with values of $p<0.05$[9].

Results

The effect of different concentrations of DMSO on the growth of 3 species of dermatophytes: *T. mentagrophytes*, *E. floccosum* and *M. canis* is given in Table 1 and Fig. 1. There was no growth in any of the 3 fungi in 10% DMSO. Between 1.25 and 5% there was a rather linear dose-related decrease in the growth of all three dermatophytes tested, significantly less than the controls ($p<0.001$ to 0.05). Between 0.15 and 0.62% there was a negligible difference from the controls in most cases, except that $p<0.05$ for the growth of *T. mentagrophytes* at DMSO 0.62% and $p<0.02$ for the growth of *M. canis* at DMSO 0.31 and 0.62% (Table 1).

The results regarding the effect on the growth of four strains of *T. rubrum* and five strains of *M. canis* are given in Table 2 and 3, as well as shown in Fig. 2 and 3, respectively. *T rubrum* was found to be slow growing with a maximum growth of 49.5 mm in controls of strain No. 3 (Table 2), more or less like *T.*

Table 1. The growth of 3 species of dermatophytes: *T. mentagrophyte*, *E. floccosum* and *M. canis* in dermasel agar containing different concentrations of DMSO (mm; mean ± SD and p values determined from Student's t test, when these means were compared with controls)

Microorganism	% DMSO in dermasel agar							
	10	5	2.5	1.25	0.62	0.31	0.15	Nil
T. mentagrophytes	0	15 ±0.82 $p<0.001$	34 ±0.82 $p<0.01$	39.25 ±1.5 $p<0.02$	40.25 ±1.71 $p<0.05$	43.25 ±1.26 $p<0.1$	45.25 ±1.71 $p>0.1$	46.5 ±0.57
E. floccosum	0	18 ±1.41 $p<0.001$	38.75 ±1.71 $p<0.01$	46 ±1.15 $p<0.05$	49 ±1.15 $p>0.1$	49.5 ±1.29 $p>0.1$	50 ±0.82 $p>0.1$	50.75 ±2.06
M. canis	0	20.75 ±2.22 $p<0.001$	33 ±1.15 $p<0.01$	47.75 ±1.71 $p<0.01$	65.25 ±1.5 $p<0.02$	66 ±0.82 $p<0.02$	68.75 ±1.5 $p>0.1$	71 ±1.15

Table 2. The growth of 4 strains of *T. rubrum* in dermasel agar containing different concentrations of DMSO (mm; mean ± SD and p values determined from Student's t test, when these means were compared with controls)

Microorganism	% DMSO in dermasel agar					
	2.5	1.25	0.62	0.31	0.15	Nil
T. rubrum (Strain-1)	12.7 ±1.5 $p<0.01$	26.8 ±2.36 $p<0.02$	27.8 ±2.06 $p<0.05$	30.75 ±1.9 $p<0.1$	34.15 ±0.5 $p<0.1$	37.75 ±0.5
T. rubrum (Strain-2)	42.3 ±3.86 $p<0.01$	47 ±1.4 $p<0.05$	47.3 ±1.9 $p>0.1$	47.75 ±0.5 $p>0.1$	48.5 ±0.82 $p>0.1$	49.25 ±0.96
T. rubrum (Strain-3)	41.3 ±1.5 $p<0.01$	46.8 ±1.26 $p<0.02$	45.8 ±1.7 $p<0.05$	48.25 ±1.7 $p>0.1$	48.8 ±1.71 $p>0.1$	49.5 ±1
T. rubrum (Strain-4)	37.5 ±5.26 $p<0.05$	44.3 ±3.1 $p>0.1$	47.5 ±2.08 $p>0.1$	47.5 ±1.29 $p>0.1$	47.25 ±1.7 $p>0.1$	47 ±1.41

Table 3. The growth of 5 strains of *M. canis* in dermasel agar containing different concentrations of DMSO (mm; mean ± SD and p values determined from Student's t test, when these means were compared with controls)

Microorganism	% DMSO in dermasel agar						
	4	2	1	0.5	0.25	0.125	Nil
M. canis (Strain-1)	28 ±2 $p<0.001$	46 ±1.8 $p<0.001$	50.75 ±2.22 $p<0.01$	55.75 ±0.96 $p<0.01$	59.25 ±3.86 $p<0.1$	64.75 ±0.96 $p>0.1$	65.5 ±1.73
M. canis (Strain-2)	29.25 ±1.71 $p<0.001$	37.75 ±0.5 $p<0.001$	44.25 ±1.26 $p<0.01$	48.75 ±1.5 $p<0.02$	51.25 ±1.26 $p<0.05$	53.5 ±1.73 $p>0.1$	56.75 ±1.71
M. canis (Strain-3)	31.5 ±1.29 $p<0.001$	44.75 ±0.96 $p<0.001$	48.5 ±1.29 $p<0.01$	54 ±1.82 $p<0.01$	58 ±2.16 $p<0.05$	62.25 ±3.9 $p>0.1$	68.25 ±1.71
M. canis (Strain-4)	26.75 ±2.36 $p<0.001$	39.75 ±3.2 $p<0.01$	49.25 ±1.5 $p<0.01$	51 ±2.17 $p<0.01$	55 ±2.83 $p<0.05$	59.5 ±1.41 $p>0.1$	64.75 ±2.06
M. canis (Strain-5)	35.25 ±1.26 $p<0.001$	40.07 ±0.96 $p<0.001$	44.5 ±1 $p<0.01$	48 ±1.4 $p<0.02$	54.25 ±1.5 $p<0.05$	60.25 ±1.26 $p>0.1$	61 ±2.16

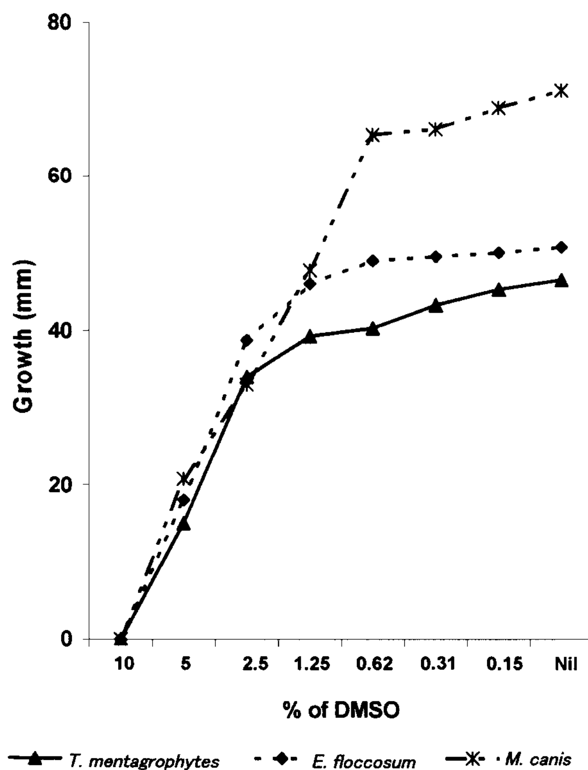

Fig. 1. The effect of different concentrations of DMSO in dermasel agar on the growth of 3 species of dermatophytes: *T. mentagrophytes*, *E. floccosum* & *M. canis*.

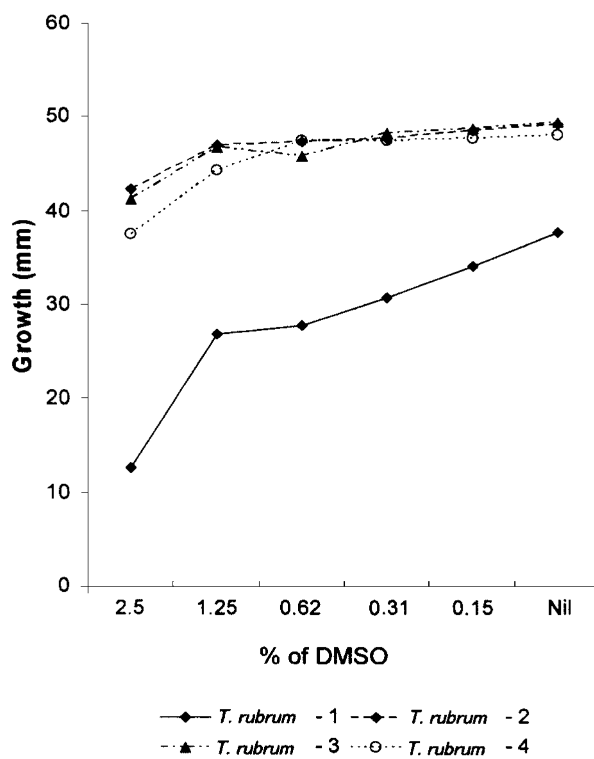

Fig. 2. The effect of different concentrations of DMSO in dermasel agar on the growth of 4 strains of *T. rubrum*.

mentagrophytes, *E. floccosum*, reaching a maximum of 46.5 and 50.75 mm, respectively (Table 1). The growth of *M. canis* was relatively faster, reaching a maximum of 71 mm (Table 1), and ranged from 56.75 to 68.25 mm in other strains (Table 3). The effect of DMSO was also

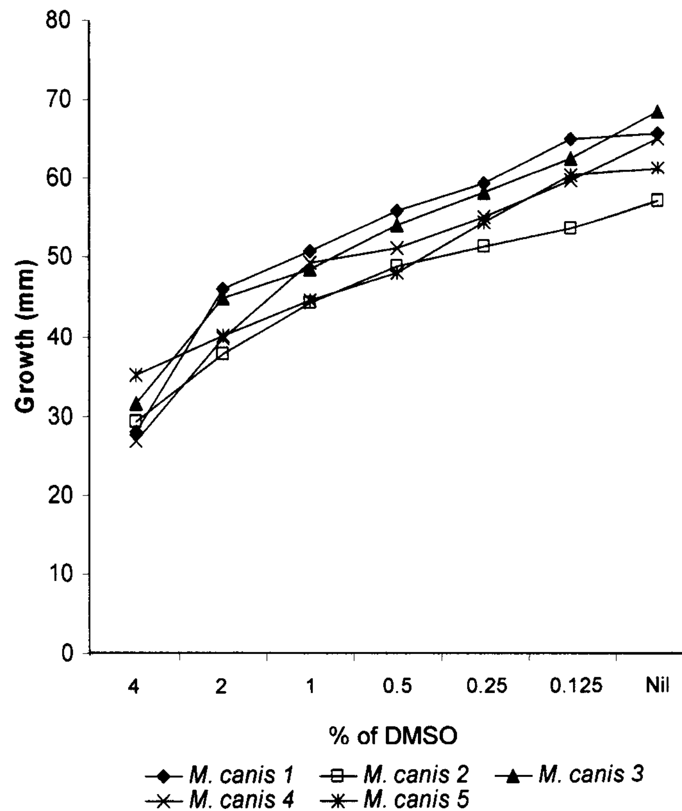

Fig. 3. The effect of different concentrations of DMSO in dermasel agar on the growth of 5 strains of *M. canis*.

relatively greater on the growth of *M. canis*. As little as 0.25% DMSO significantly inhibited the growth of different strains of *M. canis* as compared to controls (Table 3).

Discussion

DMSO is frequently used as a solvent for antifungal drugs in various studies for the determination of their MICs. The stock solutions of antifungal drugs are usually prepared in 100% DMSO and then serial dilutions are made in the culture media, which changes the concentration DMSO in different sets of plates or tubes, although the final concentration of DMSO remains 1% or below in most studies[10-12].

In the present study, DMSO 1.25 to 10% significantly affected the growth of all three dermatophytes tested. Moreover, even below DMSO 1%, the growth of *M. canis* and *T. mentagrophytes* was significantly inhibited. In the case of *M. canis*, DMSO down to 0.31 and 0.25% significantly inhibited the growth (Table 1 and 3, respectively), while *T. mentagrophytes* was significantly inhibited by DMSO 62%. That means the cut-off point of DMSO 1% may not be feasible for the comparative studies of antifungal effects of drugs against some fungi, as suggested in a previous study on yeasts (*Candida* species)[4].

In the present study the agar diffusion method was used to determine the effect of different concentrations of DMSO on dermatophytes for its simplicity, direct measurement of the growth and low cost. In a comparative study, agar-based diffusion methods are reported to be reliable alternatives to the NCCLS M27-A2 reference microdilution method. More than 96% of isolates found to be susceptible to fluconazole by the reference method were identified as susceptible by the agar based methods[13]. Because of their simplicity and low cost, the agar based diffusion methods have been used to screen the antifungal effect of herbal medicines[14].

DMSO 5 and 10% in dermasel agar were not used for studies on different strains of *T. rubrum* and *M. canis* because these concentrations showed no or very negligible effect on the growth of dermatophytes. Relatively less and slower growth of strain No. 1 of *T. rubrum* could be explained by variability in its response to DMSO. The differences in isolates, susceptibility to anti-dermatophyte activity have been reported in the literature[8, 15].

A similar inhibitory effect on the growth of dermatophytes by optical brighteners of stilbene-

disulfonic acid type has also been reported; these possibly inhibit the formation of chitin microfibrils that are essential for the normal hyphal growth and could potentiate the antimycotic effect of azoles or allylamines that interfere with the formation of ergosterol-dependent fungal cell membrane synthesis[16]. Although the effect of less than 1% DMSO is negligible on the growth of yeasts and some dermatophytes, it could perhaps have a synergistic effect of the antifungal drugs, and this needs further investigation.

In our study 10% DMSO completely inhibited the growth of dermatophytes. But DMSO is a relatively toxic substance and its topical application is able to induce both conventional irritant dermatitis and immediate non-immunological contact urticarial reaction; this latter is possibly due to release of histamine as DMSO is a very effective degranular of mast cells[6]. However, it might be a rewarding task to modify the molecular structure of DMSO to synthesize new and effective antifungal drugs.

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