

BRIEF COMMUNICATION

THE USE OF THE ANTIFUNGAL AGENT MICONAZOLE AS AN INHIBITOR OF *Blastocystis hominis* GROWTH IN *Entamoeba histolytica*/*E. dispar* CULTURES

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SUMMARY

In regions with high prevalence, *Blastocystis hominis* is frequently found in association with *Entamoeba histolytica*/*E. dispar* in xenic cultures. Its exacerbated growth is often superimposed on the growth of amebas, thus impeding the continuation of the amebas in the culture, within a few generations. The present study reports on the excellent efficacy (100%) of the antifungal agent miconazole in eliminating *B. hominis* from cultures of *E. histolytica*/*E. dispar*, thereby maintaining the integrity of the trophozoites of the amebas. Nystatin presented low efficacy (33.3%).

KEYWORDS: *Blastocystis hominis*; Miconazole; *Entamoeba histolytica*; *Entamoeba dispar*; Culture.

The human intestinal protozoan *Blastocystis hominis* causes infection that is often asymptomatic, and is found at higher prevalence in developing countries¹. Although it is one of the most common parasites encountered in stool samples, its pathogenicity is still controversial⁴. In regions of high endemicity, *B. hominis* is frequently found in xenic cultures of *Entamoeba histolytica*/*E. dispar*. In cultures it tends to grow vigorously and, unless it is eradicated completely at an early stage, it eventually overgrows and kills all amebas, usually within a few generations^{3,7}.

There are some methods for eliminating *B. hominis* from ameba cultures. DOBELL & LAIDLAW³ described ameba cyst treatment with a 0.2% solution of hydrochloric acid, at room temperature, for two hours (or with 0.1 N HCl for 10 minutes, according to CLARK & DIAMOND²), followed by washing with distilled water and reinoculation into a culture medium to which a suitable bacterial flora has been added. The acid kills *B. hominis*, fungi and bacteria, while leaving the cysts intact. In the method described by SMEDLEY⁷, ameba cultures are pelleted, resuspended in distilled water at room temperature for 20 minutes, repelleted, and then this material is inoculated into fresh culture medium. This last procedure is simple, and it is not necessary to use separate bacterial flora. However, it needs to be repeated a couple of times before the *B. hominis* is completely eliminated². The use of acriflavin (with or without subsequent passage through a medium containing lactic acid) for decontaminating the cultures is generally unsatisfactory, presenting adverse effects on the bacterial flora and, directly or indirectly, on the amebas^{2,3,7,8}. According

to SILVA⁶, culture treatment with nystatin at 25-50 UI/mL concentrations does not present high efficacy in decontamination, and *B. hominis* resistance to nystatin occurs in some cases.

This study reports on the excellent inhibitory action of the antifungal agent miconazole on *B. hominis* found in cultures of amebas, thereby keeping the amebas alive. It was approved by protocol number 217/03, from the ethical committee of Oswaldo Cruz Foundation.

Twenty fresh stool samples (from people living in the municipality of Barcelos, Amazonas State, Brazil) containing cysts of *E. histolytica*/*E. dispar* were inoculated into tubes containing 10 mL of modified Pavlova medium for polyxenic culturing⁵. The cultures were maintained at 37 °C, undergoing passage through this medium every 48 hours. Cultures positive for both amebas and *B. hominis* were treated with nystatin (Cristália, oral suspension), acriflavin (Difco Laboratories) and miconazole (miconazole nitrate, Teuto, skin lotion 2%), with the aim of eliminating the *B. hominis*. Firstly, treatment was applied using nystatin alone. This was followed by using acriflavin with nystatin in cultures that had not been decontaminated with the previous scheme. Lastly, miconazole was used against resistant *B. hominis*.

Out of 20 cultures, 18 presented *B. hominis* in association with amebas. During treatment with nystatin, two ameba cultures were lost because of high contamination by *B. hominis*. Among the remaining 16 cultures, decontamination was achieved for six of them after 2-5 treatments with nystatin at final concentrations of 25-75 UI/mL. Among

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the 10 cultures that were not freed of *B. hominis*, eight underwent treatment with nystatin and acriflavin at varying concentrations. Decontamination was not achieved via this procedure. Subsequently, they underwent miconazole treatment at final concentrations of 10-40 µg/mL, and this led to *B. hominis* elimination in all of these eight ameba cultures, after a maximum of three passages with this drug.

To evaluate the effect of miconazole alone, without interference from any other drug, we conducted this treatment procedure on two further ameba cultures. One culture was completely decontaminated after two consecutive passages, with 40 and 20 µg/mL of miconazole, respectively. For the other one, treatment was effective with just a single passage at 20 µg/mL (Table 1).

Table 1

Efficacy of drugs against *B. hominis* present in 18 ameba cultures

Drugs	No. of cultures decontaminated/ No. of cultures undergoing treatment (%)
Nystatin	6/18 (33.3%)
Nystatin + Acriflavin	0/8 (0%)
Miconazole	10/10 (100%)

In the present study, 100% efficacy (10/10) was obtained in eliminating the stages of *B. hominis*. Nystatin is currently used in the Amebiasis Laboratory of ICB/UFMG for decontaminating *E. histolytica/E. dispar* cultures in relation to *B. hominis*. However, its low yield for this purpose has already been noted previously⁶. In the present study, nystatin also demonstrated low efficacy, of 33.3% (6/18).

Due the high efficacy of miconazole in eliminating *B. hominis*, without reducing the quality of ameba trophozoites, we suggest the standardization of culturing treatments by means of the use of miconazole at a final concentration of 40 µg/mL, followed by reinforcement of the treatment in a second passage, at 20 µg/mL.

The excellent *in vitro* action of miconazole against the protozoan *B. hominis* brings the prospect that it could be routinely used in laboratories that isolate *Entamoeba* species from stools, thereby reducing problems such as loss of cultures. This procedure was shown to be effective, rapid and easy to perform. One further advantage was that treatment could be carried out directly in established polyxenic cultures, without ameba cell manipulation.

RESUMO

Uso do antifúngico miconazol como inibidor do crescimento de *Blastocystis hominis* em culturas de *Entamoeba histolytica/E. dispar*

Em regiões de alta prevalência, *Blastocystis hominis* é frequentemente encontrado em associação com *Entamoeba histolytica/E. dispar* em cultivos xênicos. Seu crescimento exacerbado se sobrepõe muitas vezes ao das amebas, impedindo a manutenção destas em cultura, dentro de poucas gerações. O presente estudo relata a excelente eficácia (100%) do antifúngico miconazol na eliminação de *B. hominis* dos cultivos de *E. histolytica/E. dispar*, mantendo-se a integridade dos trofozoítos das amebas. A nistatina apresentou eficácia baixa (33,3%).

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Received: 20 July 2006

Accepted: 18 October 2006