A CASE OF CHROMOMYCOSIS TREATED BY A COMBINATION OF CRYOTHERAPY, SHAVING, ORAL 5-FLUOROCYTOSINE, AND ORAL AMPHOTERICIN B

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Abstract. A case of chromomycosis from Comoro Islands was first treated without success with high doses of oral amphotericin B (3 g per day). Treatment with itraconazole (400 mg per day) was also unsuccessful. Then, in vitro tests were done to study the susceptibility of this Fonsecaea pedrosoi strain to antifungal drugs. It was resistant to itraconazole, sensitive to 5-fluorocytosine, and the combination of 5-fluorocytosine with amphotericin B was synergistic. The patient was then treated with this last combination of drugs, which seemed to be effective. The patient stopped this treatment after six months, and relapse occurred two years later. The best therapeutic strategy in cases of chromomycosis seems to be a combination of two drugs chosen according to the results of prior antifungal susceptibility testing.

INTRODUCTION

Chromomycosis is a subcutaneous chronic fungal disease that occurs mainly in tropical countries and is difficult to treat, particularly in long-standing cases. We report here a case of chromomycosis of more than 25 year’s duration, in a Comorian woman, who was treated with a combination of cryotherapy, shaving, and oral administration of 5-fluorocytosine (5-FC) together with amphotericin B, a combination that had been found to be synergistic. Itraconazole was ineffective.

CLINICAL DATA AND LABORATORY FINDINGS

The female patient was born in the Comoro Islands in 1932. In February 1996, Month 0, she visited family in Northern France, where she consulted the Department of Dermatology. She showed about forty dry, verrucous lesions (Figure 1) on her left foot and ankle and had some difficulty in walking. Her ankle was edematous and painful. The lesions had been developing for more than 25 years, and she had not received any prior treatment. A biopsy sample was taken from a verrucous lesion and was cut into small pieces. Direct examination and histologic study showed many typical of sclerotic or fumagoi cells (Figure 1). Parts of the biopsy sample were used for cultures in tubes containing Sabouraud’s glucose agar medium with antibiotics incubated at 25°C. Within two weeks, small black colonies appeared. This dematiaceous fungus was identified as Fonsecaea pedrosoi (Brumpt) Negroni 1936 (DF strain).

TREATMENT

As the patient was not in good health, obese, and had difficulty in walking, an at-home treatment was selected. We first administered orally a high-dose of amphotericin B, as previously recommended. The patient received 3,000 mg per day (FUNGIZONE®, four 250 mg capsules, three times daily = 30 mg/kg/d). She was checked monthly. This treatment was well tolerated. Every two months, small biopsy samples were taken for direct microscopic examination and culture. The concentration of amphotericin B was measured in serum and in an ~ 500 mg biopsy specimen by high-performance liquid chromatography. We found that the concentration of the drug was higher in the dermis (0.50 µg/g in Month 2) than in the serum (0.15 µg/mL), as shown previously. Therapeutic levels were reached only in biopsy samples and the concentration of the drug in the cutaneous lesions increased with time (0.72 µg/g in Month 4, and 0.83 µg/g in Month 6). Some of the most prominent cutaneous lesions were excised by shaving (surgical excision of lesions with a sterile scalpel maintained parallel to normal skin) under local anesthesia at each monthly consultation. In addition, the patient was kept in the hospital for three days in order to apply extensive cryotherapy to the lesions with liquid nitrogen, after local anesthesia.

Eight months later, the ankle was still edematous and painful, the remaining lesions appeared unchanged, and mycologic examinations were still positive. Oral amphotericin B was stopped and treatment started with itraconazole (SPORANOX®, 400 mg/d) in Month 9. In Month 10, although serum concentrations of itraconazole had reached a high level (1.70 µg/mL), we noticed a sudden increase in lesion severity, with a massive inflammatory reaction. Therefore, we tested in vitro the susceptibility of 3 isolates of F. pedrosoi strain from the February, April, and August biopsies, against either amphotericin B, itraconazole, 5-fluorocytosine (5-FC), or a combination of these two. Different concentrations were distributed in 55 mm Petri dishes. Suspension of fungal conidia was added. Antifungal drugs plus conidia were mixed with RPMI-1640 (supplemented with 18 mg/mL glucose, 10% decomplemented horse serum) in liquid agar. The results of these drug assays are summarized in Table 1. We found that the susceptibility of F. pedrosoi strain to these three drugs did not change over time and was resistant to itraconazole, sensitive to 5-FC, and the combination of 5-FC with amphotericin B was synergistic. In Month 11, we decided, therefore, to treat the patient with oral administration of 5-FC (ANCOTIL®, six 500 mg tablets, three times per day = 90 mg/kg/d.), in combination with oral amphotericin B (FUNGIZONE®, four 250 mg capsules, three times per day = 30 mg/kg/d). The patient was clinically and biologically checked every month. The treatment was well tolerated. The edema of the ankle resolved, the lesions flattened (Figure 1),...
FIGURE 1. A) Pretreatment cutaneous lesions of chromomycosis on the left ankle of a Comorian woman. B) Left ankle after 6 months of effective treatment (Month 16). C) Histologic study of a pretreatment biopsy sample, with brown fungal sclerotic cells.

and the patient was able to walk without pain. A biopsy sample was taken in Month 16. On direct examination, we found a small amount of brown fungal-cell debris; cultures remained negative. The treatment was continued for only one month, because the patient decided to return to the Comoro Islands. Having apparently recovered, she stopped treatment. Two years later, we learned from her family members in Dunkerque that she had had a relapse of chromomycosis in the Comoro Islands and therefore we sent the effective drug schedule to her.

DISCUSSION

The strain of *F. pedrosoi* reported in this case was highly resistant to itraconazole, both *in vivo* and *in vitro*. Other cases of chromomycosis with clinical resistance to itraconazole have been reported previously. This drug is more effective against *Cladosporium carrionii* than against *F. pedrosoi*. Itraconazole was also effective in recent cases of chromomycosis with few lesions. Furthermore, the drug susceptibility of pathogenic fungi varies geographically. In short, we have shown that: 1) in serious cases of chromomycosis due to *F. pedrosoi, in vitro* susceptibility tests are essential and must be performed early. The standard antifungal drug-testing method proposed by the National Committee for Clinical Laboratory Standards is only applicable to yeasts. No standardized drug assay for pathogenic filamentous fungi is presently available. The technique used in the present work was helpful and efficient, since *in vitro* results were well correlated with both clinical development and mycological assessment; 2) 5-FC is still one of the most efficient drugs against *F. pedrosoi*, but it should be used in combination with another antifungal drug. Some strains of *F. pedrosoi* have shown little clinical response to 5-FC alone, and resistance to 5-FC may arise during treatment; 3) oral administration of high doses of amphotericin B promotes effective levels of this fungicidal drug in the dermis; 4) the combination of 5-FC with amphotericin B is synergistic, as was previously shown experimentally. The combination of 5-FC with itraconazole has also been found to be synergistic, and was used successfully in other cases of chromomycosis; 5) oral administration of the combination of 5-FC with high doses of amphotericin B may give good clinical and mycological results, with easy administration and without toxic effects; 6) the antifungal treatment should not be stopped too early (Pradinaud and Bolzinger recommend continuing treatment during a period double that required to obtain negative results at direct examination and at fungal culture of biopsy samples and they also recommend a follow-up within five years.); and 7) removal of prominent cutaneous lesions by shaving plus cryotherapy is also very

<table>
<thead>
<tr>
<th>Antifungal drugs</th>
<th>Minimum inhibitory concentration (MIC) found* in vitro</th>
<th>Effective plasma concentration* in vivo</th>
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<tbody>
<tr>
<td>Itraconazole</td>
<td>&gt; 12.5 μg/mL</td>
<td>1 to 2 μg/mL</td>
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<td>Amphotericin B</td>
<td>between 3.12 and 6.25 μg/mL</td>
<td>0.5 to 4 μg/mL</td>
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<td>5-fluorocytosine</td>
<td>25 μg/mL</td>
<td>25 to 100 μg/mL</td>
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<tr>
<td>Itraconazole</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ 5-fluorocytosine</td>
<td>+ 12.5 μg/mL</td>
<td></td>
</tr>
<tr>
<td>Itraconazole</td>
<td>12.5 μg/mL or 3.1 μg/mL</td>
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<tr>
<td>+ Amphotericin B</td>
<td>+ 0.75 μg/mL or 3.1 μg/mL</td>
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<tr>
<td>Amphotericin B</td>
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<tr>
<td>+ 5-fluorocytosine</td>
<td>+ 12.5 μg/mL or 25 μg/mL</td>
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helpful in overcoming long-standing cases of chromomycosis.1,3,8,9

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REFERENCES