Madurella mycetomatis is highly susceptible to ravuconazole


Published in:
PLOS Neglected Tropical Diseases

DOI:
10.1371/journal.pntd.0002942

Citation for published version (APA):
Ahmed, S. A., Kloezen, W., Duncanson, F., Zijlstra, E. E., de Hoog, G. S., Fahal, A. H., & van de Sande, W. W. J. (2014). Madurella mycetomatis is highly susceptible to ravuconazole. PLOS Neglected Tropical Diseases, 8(6), e2942. DOI: 10.1371/journal.pntd.0002942
Madurella mycetomatis Is Highly Susceptible to Ravuconazole

Sarah Abdalla Ahmed1,2,3,*, Wendy Kloezen4, Frederick Duncanson5, Ed E. Zijlstra5, G. Sybren de Hoog2,3,7,8,9,10,11, Ahmed H. Fahal12, Wendy W. J. van de Sande4

1 Faculty of Medical Laboratory Sciences, University of Khartoum, Khartoum, Sudan, 2 Centralbureau voor Schimmelcultures CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands, 3 Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, The Netherlands, 4 Erasmus MC, Department of Medical Microbiology and Infectious Diseases, Rotterdam, The Netherlands, 5 Eisai Inc., Woodcliff Lake, New Jersey, United States of America, 6 Rotterdam Centre for Tropical Medicine, Rotterdam, The Netherlands, 7 Peking University Health Science Center, Research Center for Medical Mycology, Beijing, China, 8 Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University, Guangzhou, China, 9 Shanghai Institute of Medical Mycology, Changzheng Hospital, Second Military Medical University, Shanghai, China, 10 Basic Pathology Department, Federal University of Paraná State, Curitiba, Paraná, Brazil, 11 King Abdulassiz University, Jeddah, Saudi Arabia, 12 Mycetoma Research Centre, University of Khartoum, Khartoum, Sudan

Abstract

The current treatment of eumycetoma utilizing ketoconazole is unsatisfactory because of high recurrence rates, which often lead to complications and unnecessary amputations, and its comparatively high cost in endemic areas. Hence, an effective and affordable drug is required to improve therapeutic outcome. E1224 is a potent orally available, broad-spectrum triazole currently being developed for the treatment of Chagas disease. E1224 is a prodrug that is rapidly converted to ravuconazole. Plasma levels of E1224 are low and transient, and its therapeutically active moiety, ravuconazole is therapeutically active. In the present study, the in vitro activity of ravuconazole against Madurella mycetomatis, the most common etiologic agent of eumycetoma, was evaluated and compared to that of ketoconazole and itraconazole. Ravuconazole showed excellent activity with MICs ranging between ≤0.002 and 0.031 μg/ml, which were significantly lower than the MICs reported for ketoconazole and itraconazole. On the basis of our findings, E1224 with its resultant active moiety, ravuconazole, could be an effective and affordable therapeutic option for the treatment of eumycetoma.

Introduction

Mycetoma is a serious health problem with high morbidity. It is endemic in subtropical areas and often leads to severe deformity and disability [1]. The disease has long been disregarded by international health organizations but was recently recognized by WHO as a neglected tropical condition (http://www.who.int/neglected_diseases/diseases/eumycetoma). One of the main problems of eumycetoma is its recalcitrant nature, which necessitates prolonged antifungal therapy combined with massive and repeated surgical debridement. In severe cases, amputation of the affected part may be the only remaining treatment option [2]. Madurella mycetomatis is the most common fungal pathogen causing eumycetoma in arid climate zones, particularly in northeastern Africa. The infection by M. mycetomatis is characterized by the presence of black grains in tissue [3]. Previous reports showed that this fungus was most susceptible to theazole class of antifungal agents [4,5,6]. Ketoconazole and itraconazole are the most frequently used drugs for the treatment of mycetoma. However, therapy failure is common and high recurrence and amputation rates are reported [7]. Another concern is that both the Food and Drug Administration (FDA) and the European Medicines Agency (EMEA) recently restricted the use of ketoconazole due to its toxic side effects (http://www.fda.gov/Drugs/DrugSafety/ucm362415.htm) [8], making the need for an alternative treatment for eumycetoma even more urgent.

Since M. mycetomatis appeared to be most susceptible to the azole class of antifungal agents, a newazole probably has the best chance of meeting that need. A newazole currently under development is ravuconazole. Ravuconazole is a broad-spectrum triazole that showed activity against a wide array of fungal species including Aspergillus spp., Candida spp., and Cryptococcus neoformans [9,10]. Studies have shown that the efficacy of this new triazole was comparable to that of posaconazole and voriconazole [9,10,11]. In addition to antifungal activity, ravuconazole also showed in vitro activity against the parasite Trypanosoma cruzi, the
Author Summary

Madurella mycetomatis is the most common etiologic agent of eumycetoma worldwide. Treatment of this infection is very difficult and associated with high recurrence rates and low cure rates. Currently the treatment consists of a combination of surgery and antifungal therapy. Antifungal therapy is usually given for at least one year. However, the commonly used antifungal ketoconazole is too expensive for many patients in endemic countries and has many side effects. In the present study we evaluated the in vitro activity of the new antifungal agent ravuconazole against M. mycetomatis. The drug showed excellent in vitro activity against all tested strains and its prodrug, E1224, might be a potential new therapeutic option for eumycetoma caused by M. mycetomatis.

Materials and Methods

Fungal isolates

The 23 isolates were obtained from 23 patients seen at the Mycetoma Research Centre, University of Khartoum, Sudan, and preserved in the collection of Erasmus Medical Centre, Rotterdam, and CBS (Fungal Biodiversity Centre), Utrecht, The Netherlands. All the strains were previously collected and were taken from the above mentioned collections for the study. The identity of the strains was confirmed with a multi-locus analysis of rDNA internal transcribed spacer and partial large subunit and 50% of the strains, whereas 0.016 μg/ml was required to inhibit 90% of the strains. Significantly lower MICs were obtained with ravuconazole in comparison to ketoconazole and itraconazole (Mann-Whitney, p<0.0001 for both comparisons), with MICs ranging from ≤0.002 to 0.031 μg/ml (Fig. 1). Same results were obtained when using Wilcoxon’s signed rank test [Z-value: -4.1973, p-value is 0.00 for both drugs]. Moreover, there is no cross susceptibility among strains showed low MICs for ravuconazole and those of ketoconazole and itraconazole. A concentration of 0.004 μg/ml ravuconazole was needed to inhibit 50% of the strains, whereas 0.016 μg/ml was required to inhibit 90% of them.

Discussion

In this study, we demonstrated that Madurella mycetomatis, the most common etiologic pathogen for eumycetoma, was highly susceptible to ravuconazole with MICs ranging from ≤0.002 to 0.031 μg/ml. These MICs were not only considerably lower than those found for ketoconazole and itraconazole in the present study, but they were also lower than those reported for voriconazole (0.016–1 μg/ml), posaconazole (0.03–0.125 μg/ml), and isavuconazole (0.016–0.125 μg/ml) [4,5,6]. Only a few reports are available regarding the susceptibility of other eumycetoma causal agents towards ravuconazole. Studies have shown that ravuconazole has inhibitory activity against the black-grain eumycetoma species Exophiala jeaneselmei and to the saprobe Curvularia lunata that occasionally has been observed in eumycetoma [10,16]. In contrast, resistance was reported for the white-grain eumycetoma causal pathogens Pseudallescheria boydii and Fusarium species [10,16,17]. Good inhibitory activity of ravuconazole was reported for members of Chaetomium, a genus that was found to be phylogenetically close to the genus Madurella [14,18]. Low MICs were reported for Chaetomium species ranging from 0.06 to 1 μg/ml, but these values were higher than the results reported in this communication [18]. Studies of the in vitro activity of ravuconazole against the more common pathogenic fungi, including Cryptococcus neoformans, Candida species, Aspergillus species, and the
dermatophytes, showed that the drug has activity comparable to that of other triazoles [10,16,19,20]. Moreover, ravuconazole showed potent in vitro activity against the parasite *Trypanosoma cruzi* [12]. Several studies have been conducted to evaluate the in vivo efficacy of ravuconazole and E1224 using animal models of aspergillosis, candidiasis, and cryptococcosis, with each demonstrating encouraging activity of the drug [21,22,23]. In addition, phase 1/2 clinical trials have shown that ravuconazole and E1224 were well tolerated. Ravuconazole had a relatively long half-life of 4–8 days and the peak plasma concentrations of the drug ranged from 1.20 to 6.02 µg/ml when 50–400 mg/day was administrated orally for 14 days [24]. E1224 provides the advantage of more favorable pharmacokinetics with a half-life of ravuconazole (resulting from conversion of E1224 to ravuconazole) of 7.7 to 10.5 days and peak plasma levels of 3.7–379 µg/ml when 200–400 mg/day was administrated orally for 14 days [25]. This serum level of the drug is much higher than the concentration needed to inhibit 90% of the *M. mycetomatis* strains in the present study (MIC<sub>90</sub> of 0.016 µg/ml). Furthermore, in rabbits it was demonstrated that ravuconazole concentrations in the liver, adipose tissue, marrow, kidney, lung, brain and spleen exceeded concurrent plasma concentrations [26]. Moreover, high concentrations were also detected in lung and uterus of rat [27]. Due to these high levels of the drug in tissue, good therapeutic efficacy was obtained in animal models with pulmonary and disseminated aspergillosis, candidiasis, histoplasmosis, intracranial and disseminated cryptococcosis [21,23,28,29]. Based on the in vitro susceptibility generated in this study, the next step will be to study the efficacy of ravuconazole in an animal model of mycetoma.

We conclude that ravuconazole has potent in vitro activity against *M. mycetomatis*. Compared to other infectious fungi, *Madurella* is exceptionally susceptible to this drug. With its favorable pharmacokinetic properties and low toxicity, E1224 with its resultant active moiety, ravuconazole, could be a

![Figure 1. In vitro activities of ketoconazole (KTC), itraconazole (ITC), and ravuconazole (RVC) against 23 isolates of Madurella mycetomatis represented by MICs.](https://doi.org/10.1371/journal.pntd.0002942.g001)

![Table 1. In vitro susceptibility of Madurella mycetomatis to ketoconazole, itraconazole, and ravuconazole.](https://doi.org/10.1371/journal.pntd.0002942.t001)

<table>
<thead>
<tr>
<th>Antifungal agent</th>
<th>GM&lt;sup&gt;a&lt;/sup&gt; MIC (µg/ml)</th>
<th>MIC Range (µg/ml)</th>
<th>MIC&lt;sub&gt;50&lt;/sub&gt; (µg/ml)</th>
<th>MIC&lt;sub&gt;90&lt;/sub&gt; (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketoconazole</td>
<td>0.072</td>
<td>0.031–0.25</td>
<td>0.063</td>
<td>0.25</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>0.063</td>
<td>≤0.016–0.25</td>
<td>0.063</td>
<td>0.25</td>
</tr>
<tr>
<td>Ravuconazole</td>
<td>0.005</td>
<td>≤0.002–0.031</td>
<td>0.004</td>
<td>0.016</td>
</tr>
</tbody>
</table>

<sup>a</sup>GM, geometric mean.

![Table 1. In vitro susceptibility of Madurella mycetomatis to ketoconazole, itraconazole, and ravuconazole.](https://doi.org/10.1371/journal.pntd.0002942.t001)
promising antifungal agent for treatment of eumycetoma. A clinical trial is now required for an in vitro-in vivo correlation of the activity of the drug.

Acknowledgments

Ravuconazole was kindly provided by Eisai Co., Ltd. The company had no role in the study design, data collection or data analysis.

References


25. SAA WWJvdS WK AHF EEZ GSdH FD. Contribution of the paper: SAA WWJvdS WK AHF EEZ GSdH FD. Contributed reagents/materials/analysis tools: WWJvdS AHF FD. Wrote the paper: SAA WWJvdS WK AHF EEZ GSdH FD.

Author Contributions

Conceived and designed the experiments: WWJvdS WK EEZ. Performed the experiments: SAA WWJvdS WK. Analyzed the data: SAA WWJvdS. Contributed reagents/materials/analysis tools: WWJvdS AHF FD. Wrote the paper: SAA WWJvdS WK AHF EEZ GSdH FD.