Antifungal activity of itraconazole compared with hydroxy-itraconazole

in vitro

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Microbroth dilution tests in vitro showed the same IC₅₀ values for itraconazole and hydroxy-itraconazole, within a mode ± one dilution range of experimental error, for 90% of 1481 isolates of pathogenic fungi, representing 48 genera. Some 10–15% of Candida glabrata and Trichophyton mentagrophytes isolates were more susceptible to itraconazole than hydroxy-itraconazole. Replicate tests with bioassay marker strains of Candida kefyr and Candida albicans showed no susceptibility variations outside the mode ± one dilution range. We conclude that few fungi differ substantially in their susceptibility to itraconazole and hydroxy-itraconazole.

Introduction

Hydroxy-itraconazole is the antifungally active metabolite of itraconazole formed in the liver by 2-1-oxidation of itraconazole’s 1-methylpropyl substituent. The antifungal potency of itraconazole is usually assumed to be equivalent to that of hydroxy-itraconazole, although differences have sometimes been noted. Mikami et al. found they had equivalent activity against four fungi in two synthetic growth media, but itraconazole was more active than hydroxy-itraconazole in two rich culture media. A susceptibility difference between the two compounds with a bioassay marker strain of Candida kefyr has been suggested as a reason for discrepant findings in itraconazole bioassays.

For some years we have included hydroxy-itraconazole in routine antifungal susceptibility test panels with clinical isolates of fungal pathogens. We now report the activities of both compounds against a large group of fungal isolates.

Materials and methods

In total, 1481 pathogenic fungi, representing 45 genera, were originally obtained from clinical samples and identified by standard procedures. Susceptibility was tested by microplate dilution in buffered RPMI 1640 medium (Gibco-BRL, Rockville, MD, USA). Inocula from filamentous moulds were grown on Potato Dextrose agar (Difco, Detroit, MI, USA) or Sabouraud agar (Oxoid, Basingstoke, UK) incubated at 30°C until substantial numbers of conidia had formed. The surface of the thallus was scraped with a sterile wire and loose material suspended in 0.05% sodium dodecyl sulphate. Gross particles were allowed to settle, then the remaining suspensions were standardized spectrophotometrically to an O.D. of 1.0 at 570 nm. These suspensions were finally diluted 1000-fold into cultures in microdilution plates.

Microplates were incubated at 37°C for 48 h (Candida and Saccharomyces spp.) or at 30°C until control wells were visibly turbid (other fungi). For all plates, growth turbidity was measured at 405 nm, background absorbance was subtracted from the readings and the O.D. for each culture in the presence of each agent was expressed as a percentage of control absorbance. Plates in which control O.D.s were less than 0.15 were excluded from analysis. MIC was determined as IC₅₀, i.e. the lowest concentration of test compound that reduced absorbance below 50% of control. For yeasts, this end point gave the best agreement with MIC values determined by the NCCLS macrodilution reference method. The IC₅₀ values for itraconazole and hydroxy-itraconazole were extracted retrospectively from a computer database created over a 5 year period.

To investigate susceptibility differences between itraconazole and hydroxy-itraconazole for five bioassay marker yeasts, MICs with the two agents were repeated in five separate replicate tests. For each isolate, the averages of the percentage control turbidity data from the replicate tests were used to construct a dose–response curve for determination of IC₅₀.
Results

For 144 isolates, two or more replicate test results were recovered from the database. For these isolates, 84.5% of the itraconazole IC\textsubscript{50} results and 82.4% of the hydroxy-itraconazole results showed differences within two two-fold dilutions, i.e. a range of values over three two-fold dilutions. For these isolates, the geometric mean IC\textsubscript{50} values from the replicate experiments were used for comparison with the 1337 other isolates that had been tested only once.

The Table summarizes the differentials in antifungal activity measured between itraconazole and hydroxy-itraconazole. For most isolates the MICs determined for the two agents were the same within a range of three test dilutions of the compound (a maximum difference of two dilutions). From the data generated for the 144 isolates tested in replicate, 15–18% of results would be expected by chance to show MIC differences exceeding three or more dilutions owing to experimental variability. The only fungi for which more than 18% of the isolates showed differences of this magnitude between MICs of itraconazole and hydroxy-itraconazole were Candida glabrata, Trichophyton mentagrophytes and Trichophyton rubrum. For the other fungi the large MIC differences between the twoazole antifungals occurred rarely, and within the bounds of experimental variation.

Among the 206 isolates of C. glabrata, 55 (26.7%)...
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were inhibited by itraconazole at MIC values three or more dilutions lower than the hydroxy-itraconazole MIC, as compared with 16 (7.8%) isolates that were inhibited by hydroxy-itraconazole at MIC values three or more dilutions lower than the itraconazole MIC. For all seven T. mentagrophytes isolates for which MIC differentials between itraconazole and hydroxy-itraconazole were of three or more dilutions, the itraconazole MIC was lower than the hydroxy-itraconazole MIC. Among the 15 T. rubrum isolates for which the azole MICs differed by three or more dilutions, eight differences showed itraconazole as more potent than hydroxy-itraconazole and seven differences showed the opposite.

In five replicate tests with two bioassay strains of C. kefyr (SA and ATCC 46764) the itraconazole IC$_{50}$ was 0.016 mg/L and the hydroxy-itraconazole IC$_{50}$ value was 0.032 mg/L. In replicate tests with three Candida albicans bioassay strains (NCPF 3241, 3153A and RV 1488) the IC$_{50}$ values for itraconazole and hydroxy-itraconazole were all $\leq$0.008 or 0.016 mg/L.

Discussion

The results of this study indicate that, among the 1481 fungal isolates tested, 160 (10.8%) were inhibited by itraconazole and hydroxy-itraconazole at IC$_{50}$S that differed by three or more two-fold dilutions. This percentage is less than the fraction of 144 isolates tested in replicate which showed MIC variations of three or more dilutions between runs, and confirms that the antifungal potency of itraconazole and its hydroxy metabolite are essentially similar.$^{2,3}$ Approximately one-quarter of C. glabrata and T. mentagrophytes isolates were less susceptible to hydroxyitraconazole than to itraconazole, and T. rubrum isolates often differed in either direction in susceptibility to the two azoles.

In bioassays designed to measure itraconazole and hydroxy-itraconazole concentrations in clinical samples, laboratories use various marker yeast strains in the absence of a standardized method.$^6$ Since a bioassay result reflects the total antifungal activity of itraconazole + hydroxyitraconazole in a fluid sample, it may be thought more useful than a precise quantification of itraconazole alone by a more sensitive procedure such as HPLC. However, bioassays for a sample containing a mixture of the two azoles usually overestimate the true concentrations of itraconazole + hydroxy-itraconazole with itraconazole as reference standard and underestimate them with hydroxy-itraconazole as the standard.$^6$ This discrepancy might arise from susceptibility differences to the two azoles in the marker strains, differences in agar diffusion rates of the two azoles or both factors.$^{2,6}$ A previous study found that C. kefyr SA was two-fold less susceptible to itraconazole than to hydroxy-itraconazole,$^6$ whereas the present study showed a two-fold difference in the opposite direction. Differences of only two dilutions fall well within the range of normal replicate variability in this study and in the ranges for quality control yeast isolates recommended by the NCCLS,$^5$ they are therefore insufficient to permit conclusions concerning differential susceptibility of these isolates to the two azole antifungal agents. The low precision of bioassay, its relative insensitivity and the dependence of results on the choice of reference compound make bioassay at best only a semi-quantitative indicator of effective antifungal levels in an itraconazole-treated patient. Since our results show the compounds to be equipotent, HPLC measurements of both itraconazole and hydroxy-itraconazole concentrations could be reliably reported as the sum of the two concentrations to indicate bioactive levels.

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References


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