Minimal Inhibitory Concentration values of voriconazole are predictive of treatment results in murine infections by

*Aspergillus terreus* species complex

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We evaluated the efficacy of voriconazole against nine strains of *Aspergillus terreus* with different MICs (0.12 - 4 µg/ml) using a murine model. Markers of efficacy were: survival, tissue burden, galactomannan antigenemia and drug serum levels. Voriconazole was especially effective in prolonging survival and reducing the fungal load in infections by strains that showed MICs ≤ the epidemiological cut-off value (1 µg/ml). *In vitro* data might be useful for predicting the outcome of *A. terreus* infections.
Invasive aspergillosis is a frequently fatal infection that mainly affects immunosuppressed patients (1). Some species of the A. terreus complex have become increasingly important in recent years (6). Voriconazole is the recommended drug for treatment of invasive aspergillosis (16) although \textit{Aspergillus} isolates resistant to voriconazole have been reported as associated to clinical failure (5, 15). Epidemiological cut-off values (ECV) were proposed recently for several \textit{Aspergillus} spp., including \textit{A. terreus} (ECV=1 µg/ml), and those isolates showing MICs higher than ECV may have acquired resistance mechanisms (3, 10).

We have evaluated the efficacy of voriconazole in the treatment of invasive murine infection by \textit{A. terreus} species complex, testing nine clinical isolates with MICs ranging from 0.12 to 4 µg/ml (Figs. 1-3) previously determined using a reference method (2). The aim was to assess if the \textit{in vitro} data correlates with the antifungal drug efficacy.

Male OF1 mice were used in this study. All animal care procedures were supervised and approved by the Universitat Rovira i Virgili Animal Welfare and Ethics Committee. Animals were immunosuppressed 1 day prior to infection by a single intraperitoneal injection of 200 mg/kg of cyclophosphamide, plus a single intravenous injection of 150 mg/kg of 5-fluorouracil. Mice were challenged with 2x10^5 CFU via the lateral tail vein. This inoculum was suitable to produce an acute infection, with 100% of the animals dying within 13 days (data not shown).

Voriconazole was administered at 25 mg/kg of body weight/dose once a day orally (17) during 7 days. From 3 days before infection, the mice were given grapefruit juice instead of water (14). All animals received ceftazidime at 5
mg/kg subcutaneously once daily. The efficacy of voriconazole was evaluated as prolonging survival of mice, reducing tissue burden and reducing galactomannan serum levels. Groups of 8 mice were randomly established for each strain. For tissue burden studies, animals were sacrificed on day 5 post-infection and the numbers of CFU/g of kidney or brain tissue were calculated. Additionally, before being sacrificed, approximately 1 ml of blood from each mouse belonging to the tissue burden groups was extracted by cardiac puncture. Pooled serum samples were used to determine the drug concentration, by bioassay 4 h after the drug was administered (7, 11), and the galactomannan levels by enzyme immunoassay (Platelia Aspergillus®). Values were expressed as a galactomannan index (GMI) defined as the optical density of a sample divided by the optical density of a threshold serum provided in the test kit.

The Kaplan-Meier method and log rank test were used for survival studies. When multiple comparisons were carried out, the Bonferroni correction was used to avoid an increase in type I error. The tissue burden studies were analyzed using the Mann-Whitney U test. The Kolmogorov-Smirnov test was carried out to determine the normal distribution of the galactomannan serum levels and bioassay data so that they could be analysed using the T-test.

For all strains, voriconazole significantly prolonged survival with respect to the control group. For the two strains with the lowest MICs (0.12 µg/ml) survival was 100%. For the strains with MICs ≤ 1 µg/ml the survival rate of animals treated ranged from 50 to 60%. With the strain with MICs = 2 µg/ml, 25% of the infected mice survived, but in those infected with the strain with highest MIC (4 µg/ml) none of the mice survived (0%). In general, voriconazole was
Voriconazole was able to reduce the fungal load significantly in both organs tested from the animals challenged with isolates with MICs ≤1 µg/ml when compared to untreated groups and, in general, with respect to the groups infected with strains with MICs ≥2 µg/ml. For the strain with MIC = 2 µg/ml, voriconazole only reduced the fungal load in kidneys, and for the strain with MIC = 4 µg/ml there were no significant differences with respect to the control group in any of the organs studied (Figure 2).

The serum concentration of voriconazole on day 5 of the experiment was 7.01 ± 2.82 µg/ml. All serum concentrations were higher than the corresponding MICs for the strains tested (data not shown). Galactomannan serum levels were significantly lower in mice treated with voriconazole than in controls, but the galactomannan serum levels were above the cut-off for positivity (GMI > 1.5) in all cases (13) (Figure 3).

In the absence of clinical data, establishing the ECV for several Aspergillus species (3) together with the results of animal studies might contribute to the creation of clinical breakpoints for Aspergillus spp. and azoles (4). To our knowledge, this is the first study to explore the possible relationship between the MIC values for voriconazole and the outcomes of murine experimental infections by isolates of A. terreus species complex. This study demonstrates that although voriconazole is able to significantly improve the survival of animals infected with all strains tested, excellent survival rates (100%) were achieved in those mice infected with the strains having the lowest MICs (0.12...
µg/ml). However, the efficacy of voriconazole was poor in reducing the fungal load in brain of mice infected with the strains with MICs > 1 µg/ml.

A possible limitation of the present study was the inclusion of only two isolates with MICs > ECV, owing to the difficulty in finding isolates with higher MICs. Our data on galactomannan serum levels show that, in general, voriconazole worked well against all the strains of *A. terreus* tested, although in most cases the GMI was still above the threshold considered positive for invasive aspergillosis in mice (13). This could be due to the fact that voriconazole shows time-dependent fungicidal activity against some species of *Aspergillus* (8). In a previous study, galactomannan levels of mice infected with *A. terreus* and treated with posaconazole were negative at day 7 (12). Here, we only tested one dose of voriconazole because a previous pharmacokinetic study demonstrated that 25 mg/kg produced plasma levels in mice higher than in humans after therapeutic doses (17).

In this study, voriconazole MICs ≤ ECV were more predictive of *in vivo* success. Although there are only approximately 3% of the clinical isolates with MICs > ECV (9), this parameter might be important in predicting drug failure in clinical practice.

**Transparency declarations**

The authors declare no conflicts of interest

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Figure 1: Mean survival time of mice infected with *A. terreus*, VRC 25, voriconazole administered at 25 mg/kg orally once a day. Mice (n=8 per group).

\* \( P < 0.05 \) versus control;
\( ^{\circ} \) \( P < 0.05 \) versus FMR 8752;
\( ^{\circledast} \) \( P < 0.05 \) versus FMR 8806;
\( ^{\text{d}} \) \( P < 0.05 \) versus UTHSC 07-3300;
\( ^{\text{e}} \) \( P < 0.05 \) versus UTHSC 08-3714.
Figure 2. Box-plot of changes in fungal load of mice infected with $2 \times 10^5$ CFU of *A. terreus* with respect to the respective control in kidneys a) $P < 0.05$ versus control; b) $P < 0.05$ versus UTHSC 08-3714; c) $P < 0.05$ versus UTHSC 11-320; d) $P < 0.05$ versus UTHCS 07-3300; e) $P < 0.05$ versus UTHSC 10-3389; f) $P < 0.05$ versus FMR 8752; g) $P < 0.05$ versus UTHSC 11-53 and b) brain a) $P < 0.05$ versus control; b) $P < 0.05$ versus UTHSC 07-3300; c) $P < 0.05$ versus UTHSC 08-3714; d) $P < 0.05$ versus FMR 8806; e) $P < 0.05$ versus FMR 8854 of mice treated with voriconazole at 25 mg/kg orally once a day. Mice (n=8 per group).
Figure 3. Galactomannan serum levels in mice infected with *A. terreus* measured on day 5 of treatment. VRC 25, voriconazole administered at 25 mg/kg orally once a day. The horizontal line indicates the cut-off for positivity (GMI > 1.5). *P* < 0.05 versus control.
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- \( P < 0.05 \) versus UTHSC 08-3714.
Figure 2. Box-plot of changes in fungal load of mice infected with 2x10^6 CFU of _A. terreus_ with respect to the respective control in kidneys a) _a_ P < 0.05 versus control; b) P < 0.05 versus UTHSC 08-3714; c) P < 0.05 versus UTHSC 11-320; d) P < 0.05 versus UTHCS 07-3300; e) P < 0.05 versus UTHSC 10-3382; f) P < 0.05 versus FMR 8752; g) P < 0.05 versus UTHSC 11-93 and b) brain a) P < 0.05 versus control; b) P < 0.05 versus UTHSC 07-3300; c) P < 0.05 versus UTHSC 08-3714; d) P < 0.05 versus FMR 8666; e) P < 0.05 versus FMR 8854 of mice treated with voriconazole at 25 mg/kg orally once a day. Mice (n=8 per group).
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