Improving the mouse model for studying the efficacy of voriconazole

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Outbred ICR mice were rendered neutropenic, infected intravenously with Fusarium solani and treated orally with voriconazole. When given alone, voriconazole was not protective up to 40 mg/kg/day. When grapefruit juice was administered before infection, mice were protected by voriconazole. The mechanism may be inhibition of gut mucosal cytochrome enzymes that rapidly degrade voriconazole in the mouse. These murine studies support expansion of voriconazole therapy in other highly resistant systemic mycoses.

Keywords: voriconazole, mice, grapefruit juice

Introduction

Voriconazole has been developed as a broad-spectrum antifungal triazole. Like itraconazole and posaconazole, voriconazole is degraded by hepatic cytochrome enzymes. However, whereas itraconazole is degraded largely by cyp 3A4, cyp 2B9 and 2B19 are mostly responsible for the degradation of voriconazole. Clearance of voriconazole is very rapid in the mouse (terminal half-life 1 h), and generally mice have not been used successfully in studies of this antifungal. Guinea pigs, which clear voriconazole more slowly, have been the major animal models for the study of systemic mycoses. Although guinea pigs are very useful for some studies, they are costly, occupy considerable laboratory space and are not suitable for some mycoses.1 In unpublished studies, we have infected severely neutropenic guinea pigs with Fusarium solani intravenously, but have been unable to produce disease, and usually unable to recover any Fusarium from tissues of guinea pigs.

In prior studies, Sugar & Liu2 demonstrated that mice treated orally with grapefruit juice substituted for drinking water have detectable voriconazole up to 1.6 mg/L 4 h after an oral dose of 20 mg/kg/day. Furthermore, Sugar & Liu2 found that mice infected with Blastomyces dermatitidis responded clinically to treatment with voriconazole and grapefruit juice. Grapefruit juice contains naringin and 6′,7′-dihydroxy-bergamottin, both of which inhibit gut mucosal cyp 3A4. This in turn raises blood levels of drugs normally metabolized by cyp 3A4.4,5 Therefore, we considered that grapefruit juice might inhibit voriconazole metabolism by the mucosal cytochrome enzymes and thus increase intestinal absorption and systemic delivery of voriconazole. Although this mechanism has not been demonstrated specifically for voriconazole, as per Sugar & Liu,2 grapefruit juice does cause a prolonged rise in voriconazole serum concentrations.

Fusariosis is an infection associated with severe immune suppression. Treatment is difficult, as the organisms are often resistant to amphotericin B.6 In vitro studies show that Fusarium isolates tend to be susceptible to voriconazole, posaconazole and fluconazole, but not itraconazole.7 Some responses to fluconazole have been noted in mice with fusariosis, and responses to voriconazole have been noted in humans infected with Fusarium (Pfizer company files). Accordingly, we considered that grapefruit juice treatment might slow the clearance of voriconazole sufficiently for the drug to be effective in murine fusariosis.

Materials and methods

Pathogens

F. solani isolate 95-2478 was obtained from the Fungus Testing Laboratory, University of Texas Health Science Center,
San Antonio. The MIC of voriconazole for *F. solani* was 2 mg/L at 48 h incubation. Fungi were grown on potato flake agar at room temperature until used. *Fusarium* mycelial colonies were harvested by scraping the plates with sterile isotonic saline and filtering the suspension through sterile glass wool to remove hyphal fragments. Conidia were counted in a haemocytometer and suspended at the desired concentration in isotonic saline. The inocula were prepared in volumes of 0.2 mL per mouse, and confirmed by serial colony count dilutions. The viable inoculum counts are reported.

Animals

Outbred ICR mice, weighing ~25–30 g, were housed four per cage and given food and water *ad libitum*. On the day before *Fusarium* infection, mice were given a single dose of 5-fluorouracil 150 mg/kg intravenously.

Treatment

Voriconazole (obtained from Pfizer) was dissolved in polyethylene glycol. Controls were given polyethylene glycol in 0.2 mL volume once daily and also grapefruit juice. Commercially obtained bottled grapefruit juice was administered by gavage at 0.2 or 0.5 mL once daily. Administration of grapefruit juice was started 3 days before the beginning of the voriconazole therapy, or on the day voriconazole therapy began, and was continued during voriconazole administration. Treatment with voriconazole, in a volume of 0.2 mL by gavage once daily, was started 1 day before or 1 day after infection. For survival studies, the regimen was continued for 10 days after infection. Survival was observed to day 15, by which time deaths had stopped. For tissue burden studies, mice were treated to day 7. One day later, tissues were removed aseptically, homogenized and plated for serial colony count dilutions. Effort was made to keep trauma to the tissue constant.

Statistics

The logrank test was used to compare survival between treated and control animals. The Mann–Whitney test was used for comparisons of tissue burden. *P* ≤ 0.05 determined significance.

Results

In a preliminary study, at up to 40 mg/kg daily, in the absence of grapefruit juice, voriconazole did not reduce mortality in mice infected intravenously with *F. solani*. As shown in Figure 1, administration of grapefruit juice 0.5 mL/day was begun either 3 days before or on the same day voriconazole therapy commenced. Voriconazole 20 mg/kg was begun 1 day after infection. In this study of mice infected with a high inoculum, voriconazole did not prolong survival over controls. In Figure 2, mice were infected with a lower dose of *Fusarium*. Grapefruit juice administration was begun 3 days before the start of voriconazole therapy, and voriconazole therapy...
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was begun 1 day before infection. Voriconazole 10 and 20 mg/kg (P = 0.004 and 0.005, respectively) significantly prolonged survival over controls.

In tissue burden studies, mice were infected and treated with voriconazole and grapefruit juice. As shown in Figure 3, at an infecting dose of $8 \times 10^5$ cfu, kidney counts of *Fusarium* were significantly reduced by voriconazole 10 and 20 mg/kg ($P = 0.0025$ and 0.005, respectively). Spleen counts (Figure 4) were very low and not reduced further by voriconazole.

Discussion

The present study shows that voriconazole is effective in treating fusariosis in neutropenic mice. Protection was modest, but the doses we explored were limited to 10 and 20 mg/kg. MacCallum & Odds found recently that grapefruit juice substantially raises the concentration of itraconazole in mice, with levels persisting in blood for >7 h. The effect was pronounced in DBA mice, but not in BALB/c mice. This supports a specificity of mouse strain used. The effect was not observed after parenteral dosing, supporting the cause as decreased gut mucosal metabolism of itraconazole. At similar doses, Sugar & Liu have found that voriconazole persists >4 h in the blood. Although we did not measure voriconazole in the bloodstream of our grapefruit juice recipients, it is very likely that the delayed clearance of voriconazole accounted for the protective effect. Results in several studies comparing 10 and 20 mg/kg were similar, suggesting that the presence of grapefruit juice, more than the dose of voriconazole, accounts for its antifungal activity. Furthermore, the benefit may be overwhelmed by a large fungal inoculum, as no protection was shown in mice infected with $2 \times 10^6$ cfu, but protection was evident at $8 \times 10^5$ cfu.

In addition to an effect on murine cytochrome enzymes, it is possible that grapefruit juice inhibited fungal cytochrome enzymes directly. Whereas we did not test this directly *in vitro*, we included controls treated with grapefruit juice. It is also possible that the grapefruit juice stimulated some protective host immune defence. However, the timing of grapefruit juice administration (begun on the same day as, or 3 days before, voriconazole treatment) would seem very short to generate such an effect. In any case, the controls given grapefruit juice succumbed at the same rate as controls with no grapefruit juice.

These studies open the possibility of using the mouse model to evaluate voriconazole treatment in a variety of systemic mycoses. This model should also permit comparison of voriconazole with other triazoles and echinocandins, all of which are effective in some murine mycoses. As the mouse has been used most commonly for studies of systemic mycoses, and is the least costly and least sentient of the laboratory animals commonly employed, there should additionally be an impetus to use this simple model for comparison of multiple pharmaceuticals and combinations of antifungal drugs.

References


