A bioassay for determining voriconazole serum levels in patients receiving combination therapy with echinocandins

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**INTRODUCTION AND PURPOSE**

Voriconazole is widely used for the treatment of fungal infections and it is often combined with echinocandins for difficult-to-treat cases. Therapeutic drug monitoring (TDM) of voriconazole is highly recommended. Microbiological assays are simple and inexpensive techniques commonly used to measure voriconazole serum levels. Nevertheless, there are no microbiological assays for TDM of voriconazole in patients on combination therapy.

We therefore developed and validated an agar diffusion bioassay for determination of voriconazole in serum of patients on combination therapy with echinocandins.

**MATERIAL AND METHODS**

**Bioassay.** A *Candida parapsilosis* clinical isolate with CLSI MICs of voriconazole, anidulafungin, caspofungin, micafungin of 0.015, 0.25, 0.25 and 0.5 mg/L, respectively, was used. A suspension of 3x10⁵ CFU/mL was inoculated in standard medium (RPMI 1640, 0.165 MOPS, pH 7.0) with 1.5% agar, which was dispensed into plastic plates and was left to solidify. Thereafter, round wells were cut and 80μL of two-fold drug dilutions were added. Each analytical run included one blank, seven standards (0.25-8 mg/L) and two quality controls. After overnight incubation (37°C), growth inhibition was quantified by measuring the zone diameter and standard curve was created using linear regression analysis.

**Evaluation of bioassay.** Within- and between-run reproducibility was estimated by computing the coefficient of variation (CV). The effect of the presence of echinocandins in determining voriconazole levels was evaluated by spiking adequate amounts of both voriconazole and each echinocandin stock solutions into drug-free human serum and run them as unknowns.

**HPLC.** In terms of cross-validation, a previously validated high performance liquid chromatography (HPLC) method was used (Simmel F. et al *Bioanal Chem.* 2008). A total of 85 human serum samples were analyzing: 12 belong to 6 patients receiving combination therapy, 39 from 21 patients receiving voriconazole monotherapy and 34 external quality controls received from a proficiency testing program.

**Analysis.** The agreement between both methods was evaluated by linear regression analysis and Spearman’s rank correlation coefficient (r), considering HPLC as the reference method, while concordance between individual data was checked using the 2-S.5 mg/L therapeutic window (Cendejas-Bueno E. et al AAC 2013). Measurements by each methodology were performed in triplicate by two different investigators who were blinded of the results obtained with the other method.

**RESULTS**

- Voriconazole concentrations correlated linearly with the inhibition zone diameters (r²=0.98) with CV ranging from 9% to 12% (Fig. 1).
- No inhibition was observed at any concentration of anidulafungin and micafungin, while inhibition zones of 10-13.5 mm were found at caspofungin concentrations greater than 4 mg/L (data not shown).
- There was no difference between the inhibition zones of voriconazole alone and in the presence of each echinocandin in spiked human sera.
- Voriconazole levels measured by the bioassay were significantly correlated with the external quality controls (r=0.97, p<0.0001) with median % difference of -7% (range from -23 to +40%) (Fig. 2A).
- Voriconazole bioassay levels were also correlated with the HPLC results in sera from patients treated with voriconazole monotherapy (r=0.93, p<0.0001) with median % difference of 12% (range from -25 to +32%) (Fig. 2B) or combination therapy with an echinocandin (r=0.94, p<0.0001) with median % difference of 13% (range from -7 to +31%) (Fig. 2C).
- The agreement within the therapeutic window between the bioassay and the HPLC was 94%. In the remaining 6% (5/85) of the samples, the bioassay resulted in higher concentrations than the HPLC outside the upper limit of the therapeutic concentration range in patients receiving voriconazole monotherapy.

**CONCLUSIONS**

- A bioassay using a *C. parapsilosis* strain was developed for determination of voriconazole levels in serum samples.
- The bioassay was well correlated quantitatively and qualitatively with HPLC in patients treated with voriconazole alone and in combination with echinocandins.
- This is the first report of a microbiological bioassay that can be used for TDM of voriconazole in patients on combination therapy with echinocandins and may be a valid alternative tool to HPLC in clinical laboratories without specialized equipment.