Cyclosporine Enhances Fluconazole Efficacy

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Abstract

The potential exists for increased occurrence of fungal osteomyelitis following the use of immune suppressants during organ transplantation, and especially among patients who may be otherwise immunocompromised. The purpose of this study was to assess the in-vitro efficacy of fluconazole when combined with cyclosporine using a bioassay.

Ten-µL of each supernatant (fluconazole, cyclosporine and fluconazole combined with cyclosporine) was pipetted onto blank paper discs and placed in round plastic petri dishes made of yeast-nitrogen base agar, previously streaked with 4 x 10^6 cells/mL Candida albicans. Additionally, 10-µL of each supernatant was placed in Candida inoculated wells of similar plates. Zones of inhibition were recorded after 24-72 hours.

The clarity of the zones of inhibition from the combined supernatant implies that increased fungicidal activity occurred relative to fluconazole alone. This suggests that cyclosporine enhances fluconazole’s fungicidal potential and may find application for the treatment of fungal osteomyelitis in organ transplant patient population.

Keywords: cyclosporine, fluconazole, immunosuppressive agents, antifungal agent, Candida albicans, organ transplantation.

1. Introduction

Organ transplantation would not succeed without the use of immunosuppressive agents to minimize rejection. A critical disadvantage to their use is the potential for infection, particularly from Aspergillus spp. and Candida spp. It is established that mortality rates for fungi can range from 55-92% for the former and 11-81% for the latter (Singh, 2005). Immunosuppressants (cyclosporine, Cs, and tacrolimus) target calcineurin, a calcium-activated protein phosphatase (Blankenship, Wormley, Boyce, Schell, Filler, Perfect et al., 2003). Calcineurin is found in fungi and mammalian cells (Singh, 2005; Miyakawa and Mizunuma, 2007). It is responsible for virulence in fungi and regulates various cellular processes (T-cell activation, N-methyl-D-asparate [NMDA]), signaling cardiac development and hyperthropy, memory, apoptosis, cell proliferation, muscle contraction, fertility and angiogenesis, amongst others, in mammals (Blankenship et al., 2003; Miyakawa et al., 2007). Cyclosporine (Cs) and tacrolimus are also currently explored for their adjunctive antifungal activity as calcineurin inhibitors. In this study, Cs was used to simulate the cellular
environment of an immune compromised state. Therefore, the aim was to assess the \textit{in vitro} efficacy of fluconazole (FCZ) to \textit{Candida albicans} when combined with Cs.

2. Methods

2.1 Fluconazole and Cyclosporine

Supernatant solutions were prepared using the water-soluble azole, FCZ (64 µg/mL; Lot 0207K03D, Diflucan®, Pfizer, New Jersey, USA; Lot 070053, Maryna Pharma Inc., New Jersey, USA), Cs (0.4 µg/mL; Lot 958126, Cs for Injection, USP, Bedford Laboratories™, Ohio, USA), and FCZ (64 µg/mL) combined with CS (0.4 µg/mL).

2.2 Bioassay

A bioassay design was utilized. \textit{Candida albicans} (ATCC 90028) inoculum was incubated at 35°C and the turbidity was adjusted with sterile water to No. 2 McFarland standard (4 x 10⁶ cells per mL) at transmittance of 590 nm. Ten-µL of each supernatant was pipetted onto 6-mm blank paper discs (BBL™, Lot No. 5220203, 6013398 and 7222680) and then placed on the yeast-nitrogen base (YNB) agar petri dish that was previously streaked with 4 x 10⁶ cells per mL \textit{C. albicans} (Figure 1). Additionally, previously prepared 25-mL aliquots of YNB test medium were melted, allowed to cool to 48°C, inoculated with 0.5 mL of the adjusted \textit{C. albicans} suspension, then gently mixed by inversion and poured into 75-15mm round disposable petri dishes placed on a level surface. Ten-µL of each supernatant were placed in wells made in each plate (Figure 2). The zones of inhibition from both plates were recorded after 24, 48 and 72 hours.

3. Results

Zone diameters (measured after 24, 48 and 72 hours, respectively) were visibly clearer for the combined drugs versus FCZ alone and there was no zone with Cs. A similar observation was made when the same volume of each drug was placed into wells cut in the YNB agar (Figure 2) that was seeded with \textit{C. albicans} (0.8 x 10⁵ cells/mL) and zone diameters were measured after over 72 hours. The clarity in the zone diameters suggests that the combined drugs were more fungicidal relative to FCZ, which is fungistatic. The clarity of the zone of inhibition, particularly with the disks (rather than the wells) supports fungicidal activity compared with the fungistatic activity of FCZ alone.

4. Discussion

The findings were not surprising as Marchetti, Moreillon, Glauser, Bille and Sanglard (2000) previously stated that Cs, a calcineurin inhibitor, when combined with FCZ exhibited synergy, i.e. fungicidal activity. Fluconazole (FCZ) inhibits the CYP3A4 enzyme system and can potentially cause an increase in Cs levels when used concomitantly. The severity of this interaction is considered moderate with a delayed onset. Therefore in clinical practice, the serum concentration of Cs should be monitored to minimize side effects (nephrotoxicity, hypertension, and hyperlipidemia) that may occur because of reduced drug metabolism and increased serum concentration. The nephrotoxicity that can occur with Cs relates to the ability to inhibit calcineurin in the kidneys (Wu, Lai and Lien, 2004). Cyclosporine increases intracellular calcium concentration and enhances the activities of calpains and caspases (a superfamily of intracellular proteases responsible for basic and essential cellular functions (various intracellular signaling pathways)) such as apoptosis (Sorimachi, Ishiura and Suzuki, 1997; Goodsell, 2000; Stennicke and Salvesen, 2000).

Interestingly, the interaction between cyclosporine and fluconazole was also found to besynergisticallycidal (not static) on yeast was demonstrated by other researchers (Cruz, Goldstein, Blankship, Del Poeta, Davis, Cardenas et al., 2002; Onyewu, Blankenship, Poeta and Heitman (2003).
Conclusion

This study confirms the synergism, at least visually, and suggests that Cs at steady state concentrations may indeed make FCZ a formidable fungicidal agent that may be comparable to the other fungicidal agents such as amphotericin B and micafungin. Additionally, the inclusion of FCZ in a biodegradable bone delivery system in the presence of Cs should be explored as FCZ could potentially modulate fungal eradication in susceptible patient populations.

Figures

Figure 1. Zones of inhibition from disks containing solutions of cyclosporine (Cs) and fluconazole (FCZ) and cyclosporine combined with fluconazole. The efficacy of Cs-FCZ to eradicate C. albicans relative to each drug individually was reflected by the zone diameters, which were clearer and wider compared to that of Cs (no zone) and FCZ (less clear zone).
Figure 2. Zones of inhibition from wells containing solutions of cyclosporine (Cs) and fluconazole (FCZ) and cyclosporine combined with fluconazole. The efficacy of Cs combined with FCZ to eradicate *C. albicans* relative to each drug individually was reflected by the zone diameters, which were clearer but not necessarily wider, compared to that of Cs (no zone) and FCZ (less clear zone). The size of all zones decreased with time.
5. References


