Understanding antifungal prophylaxis with posaconazole in hematology patients: an evolving bedside to bench story

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Two randomized controlled clinical trials have evaluated the utility of posaconazole in antifungal prophylaxis in high-risk hematology patients.[1,2] Posaconazole use was associated with a significant reduction in fungal infections and death due to invasive fungal disease in patients undergoing induction chemotherapy for acute myelogenous leukemia or myelodysplasia (AML/MDS)[1] and those with graft-*versus*-host disease (GvHD) following allogeneic hematopoietic stem cell transplantation.[2] The strength of these trials and subsequent real life studies[3,4] led to FDA and EMA approval of posaconazole in 2007 and widespread recommendations for posaconazole use by international guidelines.[1,2,5-8] The success of posaconazole prophylaxis in the registration trials appear, however, to be at odds with the finding that serum levels of posaconazole were surprisingly low in both studies (Cavg of 583 ng/mL in AML/MDS and 1103 ng/mL in GVHD).[9,10] Furthermore, serum posaconazole levels were paradoxically lowest in the AML/MDS trial in which the most dramatic clinical effect was observed. Since the minimum inhibitory concentration (MIC) of posaconazole for most strains of *Aspergillus fumigatus* is 500 ng/mL,[11] it would appear that in the AML/MDS trial essentially half the patients in this trial should have had insufficient drug exposure to offer protection against infection. This discrepancy between serum drug levels and observed efficacy has raised new questions about our understanding of the pharmacokinetics and pharmacodynamics of posaconazole in relation to its use for primary antifungal prophylaxis.

Similar discrepancies between the antifungal effect of posaconazole and serum levels of this agent were initially noted in studies of posaconazole in animal models. In a mouse model of invasive candidiasis, treatment with a single dose of posaconazole resulted in prolonged inhibition of fungal growth for at least 20 h after the free drug concentration decreased below the MIC of the infecting strain.[12] Prolonged suppression of *C. albicans* growth was also observed even with a dose of posaconazole that did not result in serum free drug concentrations above the MIC at any point during therapy.[12] Similarly, in a study of *A. fumigatus* infection in mice, the authors noted that the drug exposure required to achieve stasis and killing of *A. fumigatus* was lower than that observed in similar studies of other triazoles.[13]

How can we resolve this apparent discrepancy between relatively low serum posaconazole levels yet documented antifungal efficacy? One explanation is that drug levels in other compartments, such as pulmonary or blood cells, are more relevant than serum for antifungal activity in primary prophylaxis. Posaconazole, like other hydrophobic agents, accumulates to much higher concentrations in tissues than in serum. For example, posaconazole concentrations in pulmonary alveolar cells have been found to be over 40-fold higher than in serum.[14] Similarly, a study of peripheral blood cells found high levels of posaconazole in mononuclear cells and leukocytes (22.5- and 7.66-fold higher than extracellular concentrations, respectively).[15] Since *Aspergillus* spores are rapidly endocytosed by pulmonary macrophages and epithelial cells after inhalation,[16,17] it is plausible that these high cell-associated drug levels could be important in mediating protection against infection.

A number of recent *in vitro* studies add support to the hypothesis that cell-associated posaconazole may be more important than free drug in mediating protection against fungal infection.[18,19] Treatment of pulmonary epithelial cells and macrophages with posaconazole *in vitro* achieved cellular concentrations of posaconazole similar to those reported in alveolar epithelial cells of posaconazole-treated patients. Notably, the posaconazole-treated cells were found to resist infection by *A. fumigatus*[14,18] The protective effect of cell-associated posaconazole persisted for at least 48 h after removal of free drug.[18] Measurement of posaconazole kinetics in epithelial cells demonstrated that this prolonged post-antifungal effect was due to the persistence of high levels of drug within the epithelial cells,[18] thus providing an explanation for the prolonged post-antifungal effect reported in animal studies of posaconazole.[12]

In addition, cellular fractionation experiments and localization studies using fluorophore-conjugated posaconazole have demonstrated that this hydrophobic antifungal accumulates only within membrane compartments of host cells, rather than throughout the whole cell.[18,19] Because membranes represent only a small fraction (<10%) of the volume of eukaryotic cells, the actual membrane concentration of posaconazole is at least 10-fold higher than prior estimates of total cellular concentration. Thus the membrane concentrations of posaconazole can reach levels as high as 400-fold greater than those found in the serum. Experiments using fluorophore-tagged posaconazole have shown that membrane-bound posaconazole transfers from the host cells to fungi.[19] In spores of *A. fumigatus*, this transfer is facilitated by the hydrophobic rodlet layer that covers these spores, and which binds avidly to posaconazole.[19] Within fungi, posaconazole also concentrates to high levels within internal membranes, including those of the endoplasmic reticulum where the posaconazole target, the enzyme CYP51a, is located.[19] This accumulation of high levels of posaconazole at the subcellular location of the target enzyme likely contributes to the antifungal efficacy of this drug.

These observations also suggest the need to reassess the importance of serum protein binding, and the role of the free drug fraction in the efficacy of posaconazole. Although posaconazole has been reported to be extensively bound by serum proteins, both bound and unbound serum posaconazole represent only a small fraction of the

total drug, as the majority of posaconazole is associated with cellular membranes. Indeed, the addition of serum to posaconazole-loaded cells does not impair their ability to inhibit the growth of *A. fumigatus in vitro*.[18] In addition, the highly hydrophobic nature of posaconazole would predict that, within the vascular compartment, this agent would easily flux between plasma proteins and the more hydrophobic cell membranes of fungi. This hypothesis is also supported by *in vitro* experimental data in which the observed antifungal effect of posaconazole in the presence of human serum is much greater than would have been predicted based on the free drug concentrations.[20] Clarifying the importance of protein binding is critically important because achievable serum free drug concentrations of posaconazole have been used to guide the selection of breakpoints for the identification of resistance to this agent when performing antifungal susceptibility testing.[13]

Implications for therapeutic drug monitoring and dosing strategies

The results of these *in vitro*, animal, and clinical studies are beginning to shed new light on our understanding of the mechanisms of action and efficacy of posaconazole in primary antifungal prophylaxis, and suggest the need to rethink our strategies for use of serum therapeutic drug monitoring in this setting. Studies of human and animal pharmacokinetic data for posaconazole have focused on serum levels, and there are no data available on the pharmacokinetics of membrane posaconazole in either of these populations. The prolonged antifungal effect observed in animals and the posaconazole cellular pharmacokinetic studies *in vitro* suggest that membrane-associated posaconazole persists long after serum levels decline, and is able to confer protection against infection. Studies are needed in both animals and patients to confirm these findings and to guide the optimal use of this agent. By extension, the available data suggest that the ability of serum drug measurements to identify patients with inadequate posaconazole membrane concentrations is likely to be limited. The development of a therapeutic test for membrane-associated posaconazole will be invaluable for better monitoring of patients, and also for guiding dosing strategies for the new tablet and intravenous formulations of posaconazole that are currently in late stage clinical trials.

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References

1. Cornely OA, Maertens J, Winston DJ, Perfect J, Ullmann AJ, Walsh TJ, et al. Posaconazole vs. fluconazole or itraconazole prophylaxis in patients with neutropenia. N Engl J Med. 2007;356(4):348-59.
2. Ullmann AJ, Lipton JH, Vesole DH, Chandrasekar P, Langston A, Tarantolo SR, et al. Posaconazole or fluconazole for prophylaxis in severe graft-versus-host disease. N Engl J Med. 2007;356(4):335-47.
3. Girmenia C, Frustaci AM, Gentile G, Minotti C, Cartoni C, Capria S, et al. Posaconazole prophylaxis during front-line chemotherapy of acute myeloid leukemia: a single-center, real-life experience. Haematologica. 2012;97(4):560-7.
4. Vehreschild JJ, Ruping MJ, Wisplinghoff H, Farowski F, Steinbach A, Sims R, et al. Clinical effectiveness of posaconazole prophylaxis in patients with acute myelogenous leukaemia (AML): a 6 year experience of the Cologne AML cohort. J Antimicrob Chemother. 2010;65(7):1466-71.
5. Walsh TJ, Anaissie EJ, Denning DW, Herbrecht R, Kontoyiannis DP, Marr KA, et al. Treatment of aspergillosis: clinical practice guidelines of the Infectious Diseases Society of America. Clin Infect Dis. 2008;46(3):327-60.
6. Maertens J, Marchetti O, Herbrecht R, Cornely OA, Fluckiger U, Frere P, et al. European guidelines for antifungal management in leukemia and hematopoietic stem cell transplant recipients: summary of the ECIL 3--2009 update. Bone Marrow Transplant. 2011;46 (5):709-18.
7. Pagano L, Lumb J. Update on invasive fungal disease. Future Microbiol. 2011;6(9):985-9.
8. Skiada A, Lanternier F, Groll AH, Pagano L, Zimmerli S, Herbrecht R, et al. Diagnosis and treatment of mucormycosis in patients with hematological malignancies: guidelines from the 3rd European Conference on Infections in Leukemia (ECIL 3). Haematologica. 2013;98(4):492-504.
9. Jang SH, Colangelo PM, Gobburu JV. Exposure-response of posaconazole used for prophylaxis against invasive fungal infections: evaluating the need to adjust doses based on drug concentrations in plasma. Clin Pharmacol Ther. 2010;88(1):115-9.
10. Kontoyiannis DP, Lewis RE. How I treat mucormycosis. Blood. 2011;118(5):1216-24.
11. Sabatelli F, Patel R, Mann PA, Mendrick CA, Norris CC, Hare R, et al. In vitro activities of posaconazole, fluconazole, itraconazole, voriconazole, and amphotericin B against a large collection of clinically important molds and yeasts. Antimicrob Agents Chemother. 2006;50(6):2009-15.
12. Andes D, Marchillo K, Conklin R, Krishna G, Ezzet F, Cacciapuoti A, et al. Pharmacodynamics of a new triazole, posaconazole, in a murine model of disseminated candidiasis. Antimicrob Agents Chemother. 2004;48(1):137-42.
13. Lepak AJ, Marchillo K, Vanhecker J, Andes DR. Posaconazole pharmacodynamic target determination against wild-type and Cyp51 mutant isolates of Aspergillus fumigatus in an in vivo model of invasive pulmonary aspergillosis. Antimicrob Agents Chemother. 2013;57(1):579-85.
14. Conte JE Jr, Golden JA, Krishna G, McIver M, Little E, Zurlinden E. Intrapulmonary pharmacokinetics and pharmacodynamics of posaconazole at steady state in healthy subjects. Antimicrob Agents Chemother. 2009;53(2):703-7.
15. Farowski F, Cornely OA, Vehreschild JJ, Hartmann P, Bauer T, Steinbach A, et al. Intracellular Concentrations of Posaconazole in Different Compartments of Peripheral Blood. Antimicrob Agents Chemother. 2010;54(7):2928-31.
16. Wasylnka JA, Moore MM. Uptake of Aspergillus fumigatus Conidia by phagocytic and nonphagocytic cells in vitro: quantitation using strains expressing green fluorescent protein. Infect Immun. 2002;70(6):3156-63.
17. Filler SG, Sheppard DC. Fungal invasion of normally non-phagocytic host cells. PLoS Pathog. 2006;2(12):e129.
18. Campoli P, Al Abdallah Q, Robitaille R, Solis NV, Fielhaber JA, Kristof AS, et al. Concentration of antifungal agents within host cell membranes: a new paradigm governing the efficacy of prophylaxis. Antimicrob Agents Chemother. 2011;55(12):5732-9.
19. Campoli P, Perlin DS, Kristof AS, White TC, Filler SG, Sheppard DC. Pharmacokinetics of posaconazole within epithelial cells and fungi: Insights into potential mechanisms of action during treatment and prophylaxis. J Infect Dis. 2013;208(10):1717-28.
20. Lignell A, Lowdin E, Cars O, Chryssanthou E, Sjolin J. Posaconazole in Human Serum: a Greater Pharmacodynamic Effect than Predicted by the Non-Protein-Bound Serum Concentration. Antimicrob Agents Chemother. 2011;55(7):3099-104.