Fluconazole Penetration into the Human Prostate

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Fluconazole concentrations in the serum and prostate of human volunteers undergoing transurethral resection for benign prostatic hypertrophy were measured. There was a high correlation (r = 0.783) between serum (mean = 6.6 μg/ml) and tissue (mean = 1.9 μg/g) fluconazole concentrations, and these data were used to construct a model for local tissue concentrations.

The prostate has been reported to be a nidus for persistent infection following treatment for systemic Cryptococcus neoformans infection, particularly in the immunocompromised host (6, 7, 13). Fluconazole is currently an accepted therapy for systemic cryptococcal infections, but there is no published information on fluconazole penetration into the human prostate. To address this important question, we measured steady-state fluconazole concentrations in prostatic tissue obtained by transurethral prostatic resection and in serum from human volunteers. Factors influencing the tissue distribution of fluconazole into the extracellular and intracellular compartments are analyzed, and the implications for the treatment of cryptococcal prostatitis are discussed.

The study was designed as an open-label, unblinded investigation. Otherwise healthy patients with benign prostatic hypertrophy who were scheduled for elective transurethral prostatic resection at our affiliated Department of Veterans Affairs Hospital were enrolled. All subjects screened for study inclusion consented to participate. Patients received 400 mg of oral fluconazole on day 1 and then 200 mg of oral fluconazole per day on days 2 through 5. Patients were instructed to take the daily dose at 2000 h. Patients were admitted to the hospital on day 5. Blood for determination of serum fluconazole concentrations was collected immediately before the final dose (trough), 2 h after the final dose (peak), and during surgery on day 6. Surgery was performed 12 to 15 h after the last dose. Informed consent was obtained from all patients prior to study participation. The protocol was evaluated and approved by the Institutional Review Board of the University of Mississippi Medical Center and follows the guidelines for human experimentation of the U.S. Department of Health and Human Services.

Blood specimens were centrifuged at 500 × g for 10 min. The serum was separated within 1 h of collection and frozen at −70°C until drug analysis. Prostatic tissue was collected during the transurethral resection and immediately frozen at −70°C. Prior to the determination of drug concentrations, the tissue was weighed, freeze-thawed three times, and extracted with chloroform. Fluconazole concentrations in the sera and tissue extracts (performed by the Fungal Testing Laboratory, University of Texas Health Sciences Center at San Antonio) were determined by megabore capillary gas-liquid chromatography as previously described (5). Fluconazole concentrations are expressed as micrograms per milliliter of serum and micrograms per gram of tissue. Linearity of this assay extends from 0.2 to 200 μg/ml. The assay had within-run and run-to-run coefficients of variation of 8.1% (n = 10) and 9.9% (n = 10), respectively, at a concentration of 2.0 μg/ml and of 6.3% (n = 10) and 4.5% (n = 10), respectively, at a concentration of 7.0 μg/ml. Regression analysis relating the serum and prostate drug concentrations was performed with Sigma Plot software (Jandel Scientific, San Rafael, Calif.) by the Cholesky decomposition algorithm.

The amount of blood and urine contaminating the prostatic tissue could significantly affect measured fluconazole concentrations. We therefore estimated the degree of contamination by measuring the amount of hemoglobin contained in supernatants of tissue homogenates and expressed the results as microliters of blood per gram of tissue. Hemoglobin concentrations in the supernatants was measured by the cyanmethemoglobin spectrophotometric absorption assay (9). The sensitivity of the assay allowed the detection of 5 μl of blood per g of tissue. Evaluation of urine contamination was estimated by measuring creatinine (detection limit = 8.84 μM) and urea nitrogen (detection limit = 0.7 mM) in tissue supernatants by our clinical laboratory (ACA IV autoanalyzer; Dupont). Estimates of the amount of blood contaminating the samples and contributing to the measured fluconazole revealed consistently less than 5% contamination (3.75% ± 1.59% [mean ± standard deviation]). In addition, urea and creatinine concentrations were below detectable limits in all samples, and urine contamination was therefore considered insignificant.

Tissue drug concentrations were estimated by a simple ratio method and by a previously described tissue model (10, 12). The estimated prostatic distribution of fluconazole based on a simple ratio method was calculated by dividing the concentration in prostatic tissue by the concentration in serum to yield the percent distribution. Prostatic tissue penetration was also calculated on the basis of a tissue model where extracellular fluid was estimated to be 16%, serum was estimated to be 4%, intracellular fluid was estimated to be 54%, and solid tissue mass was estimated to be 26% of tissue weight (10). This allows a more precise localization of the drug in the intracellular or extracellular compartment but requires additional information or assumptions concerning intracellular penetration. Since data concerning the intracellular penetration of fluconazole into cells are limited, we expressed the extracellular fluconazole concentration as a function of the intracellular penetration throughout a range of possible values. The equation for this function is given in the legend to Fig. 2.

Ten patients were enrolled in the study, but data were collected for only eight patients. Two patients had inadequate quantities of tissue available for pathological study and com-
pletion of drug analysis. Concentrations of fluconazole in serum and tissue showed significant interpatient variation (Table 1). There was a strong correlation \((r = 0.783)\) between the drug concentrations in the serum and the tissue of individual patients, except for patient number eight, who showed high drug concentrations in serum but a very low drug concentration in tissue. When this patient was included in the data analysis, the correlation coefficient dropped to 0.488, and the regression line deviated far from the origin (Fig. 1). In addition, the datum point for patient 8 lies outside the 99% confidence interval of the regression for the other datum points. Therefore, this patient was considered an outlier and removed from the remaining data analysis.

The average fluconazole distribution in tissue by the ratio method was 29%. The proportion of tissue fluconazole in the extracellular space is a function of the intracellular penetration of the drug, for which little information is available. We therefore decided to plot the extracellular fluconazole concentration over a range of values of the intracellular penetration to more clearly demonstrate the relationship between the two (Fig. 2). The extracellular fluconazole concentration is expressed as a percentage of the serum drug concentration. The intracellular fluconazole penetration is expressed as a ratio of the intracellular concentration to the extracellular concentration. If there is no intracellular penetration, fluconazole reaches a higher concentration in the extracellular space than in the serum (157%). As the intracellular proportion of the drug increases, the extracellular concentration must decrease. At a 1:1 ratio, the extracellular concentration has decreased to 36% of the level in serum, and at a 2:1 ratio it is 20% of the level in serum. Independent data on fluconazole intracellular penetration are required to localize a point on the curve.

Involvement of the prostate in \(C.\ neoformans\) infection was first reported in 1946 as a granulomatous complication of disseminated disease (15). Host defenses are limited within the prostate, and it is a nidus for persistent infection. The infection may present urinary tract symptoms (e.g., urinary retention) or it may be relatively asymptomatic. Treatment is complicated by the fact that the prostate is a privileged site with poor penetration by many antimicrobial agents (3). Relapses after therapy with fluconazole or amphotericin B and persistence of \(C.\ neoformans\) in the urine and prostatic fluid of human immunodeficiency virus-positive patients have raised concern about the efficacy of therapy at this site of infection. Larsen et al. (7)
hypothesized that prostatic tissue may be a sequestered reservoir of infection even in patients who respond to routine therapy for *C. neoformans* meningitis.

One would like to compare the MIC for the infectious agent with the concentration of the antimicrobial agent of interest at the site of infection. For fluconazole, a range of MICs of 0.37 to 6.25 μg/ml was reported for *C. neoformans* isolates collected from primary cases on initial presentation (4). There are, however, isolates of *C. neoformans* for which MICs are ≥20 μg/ml (1). The mean total concentration of prostatic fluconazole in our patients was 1.93 μg/ml, or 29% of the concurrent serum value. Similarly, penetration of fluconazole into rabbit prostate tissue was reported to be approximately 30% of the concentration in serum (11a). This datum by itself is of little value, and ultimately only clinical studies can answer the question of efficacy. One may speculate that the intracellular or extracellular localization of the drug is of importance depending on the localization of the infectious agent. *C. neoformans* growth takes place predominately in the extracellular milieu. One would therefore expect extracellular concentrations of fluconazole in the prostate to be of primary interest. Extracellular concentrations of other water-soluble agents, specifically cefuroxime and ampicillin (12), have been shown to be underestimated by simple ratios of tissue homogenates to sera, probably because of the poor intracellular penetration of beta-lactam antibiotics. If fluconazole behaves in the same way as these antibiotics, we would expect low intracellular concentrations and correspondingly high extracellular concentrations (on the order of 100 to 150% of concentrations in serum). For the range of concentrations in serum achieved in our patients, this would imply extracellular concentrations greater than the MICs for most sensitive cryptococcal isolates. However, the only published data on intracellular fluconazole penetration (in polymorphonuclear leukocytes and McCoy cells) suggest intracellular/extracellular fluconazole ratios in the range of 1.3 to 2.2 (11). In our model, this would lead to extracellular fluconazole concentrations between 18 and 33% of the concentration in serum. In this case, the extracellular fluconazole concentration would be lower than the MICs for a significant number of even the sensitive cryptococcal isolates. These calculations suggest that a 200-mg/day dosage of fluconazole at best marginally inhibits *C. neoformans* infection in the prostate. This conclusion is supported by the clinical reports of both success and failure in the use of fluconazole against this infection (2, 6, 8, 14). Theoretical information must be interpreted with caution since there is a poor correlation between antifungal MICs and efficacy of therapy with fluconazole. The effects of inflammation on the ability of antimicrobial agents to penetrate the prostate is also unknown, and many other factors play important roles in the outcome. With these caveats in mind, we note that the results of this study and the clinical information available suggest that higher dosages of fluconazole (i.e., >200 mg/day) for a prolonged period may be needed in treating known cryptococcal prostatitis. Clinical studies are necessary to definitively answer this question.

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REFERENCES