Fluconazole Distribution to the Brain: A Crossover Study in Freely-moving Rats Using In Vivo Microdialysis

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Received June 24, 1996; accepted July 29, 1996

Purpose. The purpose of this study was to determine if the microdialysis sampling technique is feasible to study the central nervous system distributional kinetics of a novel triazole antifungal agent, fluconazole, in an awake, freely-moving rat model, and to determine fluconazole distribution to the extracellular fluid (ECF) of the brain.

Methods. The relative recovery of the microdialysis probes (CMA-12) was determined in vitro and in vivo by retrodialysis using UK-54,373, a fluorinated analog of fluconazole. Sprague-Dawley rats received 10 mg/kg and 20 mg/kg fluconazole IV bolus doses in a crossover design, and brain extracellular fluid fluconazole concentrations were monitored using microdialysis and on-line HPLC analysis. The plasma fluconazole concentration vs. time data were determined using sequential blood sampling and HPLC analysis.

Results. There was no statistical difference between relative probe recoveries for both fluconazole and UK-54,373, either in vitro or in vivo, and probe recoveries did not change during the course of the in vivo crossover experiment. Fluconazole rapidly distributes into the brain ECF and the average brain distribution coefficient (brain/plasma AUC ratio) was 0.60 ± 0.18 and was independent of dose. Plasma pharmacokinetic parameters were linear in the dose range studied.

Conclusions. Fluconazole rapidly reaches a distribution equilibrium between brain extracellular fluid and plasma, and the distribution to the brain is substantial and not dependent on dose over a two-fold range. Furthermore, the results indicate that microdialysis utilizing UK-54,373 as the in vivo retrodialysis probe calibrator is a feasible method to study the transport of fluconazole into the central nervous system.

KEY WORDS: microdialysis; fluconazole; pharmacokinetics; brain distribution.

INTRODUCTION

The incidence of invasive fungal infections in the central nervous system has increased in recent years, due in large part to the increased patient population which is immunocompromised (AIDS patients, organ transplant patients and patients undergoing chemotherapy). These infections are extremely difficult to treat and have a poor prognosis. Most antifungal agents, such as amphotericin B, ketoconazole, and itraconazole, do not penetrate well into the central nervous system (CNS), which may limit therapeutic efficacy (1,2). Fluconazole readily penetrates into the CNS, but its use is limited by high relapse rates and the emergence of resistance (3,4). Fluconazole is a synthetic triazole antifungal agent which is different from other antifungal agents in that it has a greater relative penetration into the CNS (2). Fluconazole has moderate lipophilicity (log P<sub>octanol</sub> = 0.5) and it is unionized in the blood (5). These characteristics, when combined with low protein binding, are responsible for fluconazole's good tissue penetration. Its therapeutic efficacy in treating cryptococcal and coccidioidal meningitis has been established in clinical studies (6,7), and the therapeutic failure rate is approximately 30 to 50% (8). One possible reason for the limited efficacy is that the fluconazole concentrations in the CNS have not reached an effective concentration. In vitro tests have suggested that the antifungal potency of fluconazole is relatively weak, with the MIC ranging from 0.0625 to 50 μg/ml or higher for cryptococcus (9–11). The concentration-response curve of fluconazole shows that increasing fluconazole concentration will increase the extent of fungal inhibition (10). These results suggest that high fluconazole concentrations at the infection site are essential to achieve therapeutic efficacy. Therefore, increasing the fluconazole concentrations in the CNS may lead to greater success in treating invasive fungal infections in the CNS. However, the results of high dose fluconazole in treating invasive fungal infections in the central nervous system are controversial (6,11,12), and the mechanisms of fluconazole transport across blood-brain barrier remain unclear. The transport of fluconazole into the CNS can not be fully explained by simple passive diffusion. Fluconazole rapidly enters the CSF, and the free concentration in the CSF is significantly less than the free concentration in plasma at steady state (1). These properties may be indicative of an efflux transport system at the choroid plexus or blood-brain barrier. Further elucidation of the transport process of fluconazole across blood-brain barrier is critical in enhancing the use of this drug in fungal meningitis.

Several techniques have been employed to study drug transport to the brain. The brain tissue homogenate technique is one of the traditional methods used to determine drug distribution to the brain. However, only one concentration-time point can be obtained for one animal when using this technique. In order to obtain the entire brain drug concentration-time profile, many animals must be used and interanimal variability often obscures the result. The measured drug concentration is actually the averaged total drug concentration in brain cells, extracellular fluid, CSF and retained blood, and it is difficult to interpret these data regarding transport across the blood-brain barrier.

Microdialysis has recently been used in neuropharmacokinetic studies (13–15). When compared to the traditional tissue sampling techniques, brain microdialysis coupled with on-line HPLC analysis provides a powerful tool to continuously monitor the brain drug concentration of animals for pharmacokinetic purposes. Frequent determinations may be made, which can provide more information about the shape of the concentration-time profile of the drug, without removing any fluid from the tissue. Determination of the free drug concentration on both sides of the blood-brain barrier is important for characterizing the transport processes across blood-brain barrier.

The objective of this study was to determine brain ECF fluconazole concentrations and CNS distributional parameters using the intracerebral microdialysis technique. Microdialysis probe recoveries were determined using in vivo retrodialysis. The transport of fluconazole across the blood-brain barrier was studied following 10 and 20 mg/kg IV bolus doses to an awake, freely-moving rat model.

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Fluconazole Distribution to the Brain Using Microdialysis

MATERIALS AND METHODS

Determination of Probe Recovery by Gain and Loss In Vitro

(1) Recovery by Gain (RG) In Vitro

A CMA-12 3mm microdialysis probe (CMA-Microdialysis, Acton, MA) was placed in a 2 ml vial containing well-stirred artificial cerebrospinal fluid (pH 7.4) at 37°C containing fluconazole or UK-54,373. The probe was perfused with drug-free artificial CSF at a flow rate of 0.5 μl/min from a gas-tight syringe using a Harvard 33 syringe pump (Harvard Apparatus, Natick, MA) and the concentration of fluconazole and UK-54,373 in the perfusate (Cout) was determined by on-line HPLC. The fluconazole or UK-54,373 concentration (Cm) in the medium surrounding the microdialysis probe was analyzed by HPLC. The relative recovery by gain (RG) in vitro was calculated as:

\[ \text{RG} = \frac{\text{Cout}}{\text{Cm}} \]  

(1)

(2) Recovery by Loss (RL) In Vitro

The same probe was then placed in a 2 ml vial containing drug-free artificial CSF. Artificial CSF containing fluconazole or UK-54,373 was placed into the gas-tight syringe and perfused through the probe at a flow rate of 0.5 μl/min. Per fusate was analyzed by on-line HPLC to determine fluconazole or UK-54,373 concentration (Cout). The fluconazole or UK-54,373 concentration in the perfusate (Cm) was determined and the recovery by loss (RL, retrodialysis) for both fluconazole and UK-54,373 was calculated as follows:

\[ \text{RL} = \frac{(\text{Cin} - \text{Cout})}{\text{Cin}} \]  

(2)

Animal Surgery

Male Sprague-Dawley rats weighing between 250–350 g were used in this study. At all times, including the microdialysis sampling period, the rats had free access to food and water. Surgical preparation of these rats was done using aseptic technique, and all surgical procedures were performed under anesthesia using an i.p. dose of 50 mg/kg sodium pentobarbital (Abbott Laboratories, Chicago, IL 60064). An i.m. dose of 60,000 units procaine penicillin G (Wyeth-Ayerst) was given following surgery.

Microdialysis guide cannulae (for CMA-12 probes) were stereotaxically placed in the frontal cortex of the brain (P + 4.2 mm, M-2.0 mm using bregma as the reference, and 2.1 mm below the brain surface) and the rat was allowed to recover for one week. The femoral artery and vein were surgically exposed and separated from surrounding tissues, and cannulated using PE-10 connected to PE-50 tubing. A 40 unit/ml heparinized saline solution was maintained in the arterial cannula to prevent clotting. The CMA-12 microdialysis probe was slowly introduced into the cortex through the microdialysis guide cannula immediately after blood vessel cannulation, and was then perfused at 0.5 μl/min with artificial CSF containing approximately 1 μg/ml UK-54,373 as the in vivo probe calibrator. These procedures adhered to the "Principles of Animal Care" outlined by NIH publication #85-23, and were approved by the Institutional Animal Care and Use Committee of the University of Nebraska Medical Center.

Recovery by Loss (RL) of Fluconazole and UK-54,373 In Vivo

Four male Sprague-Dawley rats were used in this study. Each rat had a CMA-12 3 mm microdialysis probe placed in the frontal cortex, and the probe was immediately perfused with artificial CSF containing fluconazole (approximately 1 μg/ml) and UK-54,373 (approximately 1 μg/ml), at 0.5 μl/min. The perfusate leaving the brain probe was collected directly into the 10 μl injection loop, and injected into the HPLC at 20 minute intervals to determine the concentration of fluconazole and UK-54,373 in the microdialysate (Cout). After the in vivo experiment, the fluconazole and UK-54,373 concentration in the perfusate entering the brain probe (Cin) was determined. The retrodialysis recoveries (RL) of fluconazole and UK-54,373 were then calculated according to equation 3 (see data analysis).

Fluconazole Brain Distribution Studies

Fluconazole doses of 10 mg/kg and 20 mg/kg were administered by intravenous bolus to six rats in a balanced crossover design. Rats 1–3 received 10 mg/kg fluconazole in the phase one and 20 mg/kg fluconazole in the phase two and rats 4–6 received the opposite treatment order. The washout interval between the two doses was approximately 48 hours (6–8 elimination half-lives), to ensure the first fluconazole dose had been completely eliminated from the rats. Cortical extracellular fluid was continuously sampled by microdialysis for over 20 hours (approximately 3–4 half-lives) and the fluconazole concentration in the microdialysate was determined by on-line HPLC. In vivo probe recovery was determined by measuring the loss of UK-54,373 from the dialysate, and was calculated according to equation 3. Blood samples (0.3 ml) were obtained from the arterial cannula at various time points after dosing, and the plasma was harvested and stored frozen at −20°C until analysis.

Sample Analysis

(1) Analysis of Plasma Sample

Fluconazole plasma concentrations were determined by HPLC with UV detection and UK-51,060, an analog of fluconazole, was used as the internal standard. Plasma samples (100 μl) were extracted with 5 ml of methylene chloride and centrifuged at 1000 g for 15 minutes. The organic phase was transferred to another test tube and evaporated by passing a gentle stream of nitrogen over the methylene chloride. Residues were reconstituted in 100 μl of mobile phase, and a 20 μl aliquot was injected into the Shimadzu HPLC system, which consisted of a LC-6A pump, a SPD-10AV UV-VIS spectrophotometric detector, a SIL-9A auto-injector. Peak heights were determined with a CR-601 integrator, and a peak height ratio vs. concentration calibration curve was used to quantify unknowns. Separation was performed on a HP ODS 2.1 x 200 mm, 5-μm column, and analytes were eluted by a mixture of 0.02 M ammonium monobasic phosphate, adjusted to pH 7 with 5 N sodium