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

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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 in 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 distributional 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). Flucytosine 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 $P_{octanol}$ = 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 the 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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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:

$$RG = Cout/Cm \quad (1)$$

(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. Perfusate 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 (Cin) was determined and the recovery by loss (RL, retrodialysis) for both fluconazole and UK-54,373 was calculated as follows:

$$RL = (Cin - Cout)/Cin \quad (2)$$

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 × 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

hydroxide, and acetonitrile (87:13 w/w). All analyses were carried out with a detection wavelength of 210 nm and a mobile phase flow rate of 0.5 ml/min. The calibration curve was linear in the fluconazole concentration range of 0.3–30 μg/ml. The fraction unbound in plasma was estimated using ultrafiltration followed by HPLC analysis.

(2) On-line Analysis of Microdialysis Samples

Microdialysis samples from the brain probe effluent were collected directly into 10-μl matched injection loops over a collection interval of 20 minutes. The Shimadzu HPLC system used here consisted of a LC-10AD pump, a CR501 integrator, and a SPD-10AV spectrophotometric detector. A HP ODS 2.1 × 200 mm, 5-μm column was used for separation, and analytes were eluted by the mobile phase described above. The time program for the collection interval, loop switching, sample injection, and to start the integrator was controlled by using a Valco digital valve sequence programmer model VDSP2 (Valco Instruments Co. Inc., TX). The injection loops were fitted into a 10-port Valco valve model E36 (Valco Instruments Co. Inc., TX). All on-line analyses were carried out with a detection wavelength of 210 nm and a mobile phase flow rate of 0.5 ml/min.

Data Analysis

The *in vivo* probe recovery was determined by estimating the loss (the extraction ratio) of the fluorinated analog (UK-54,373), which was calculated from the concentration in the dialysate (the concentration leaving the probe, Cout) relative to the concentration of the analog in the perfusate (the concentration entering the probe, Cin) as expressed in the following equation:

$$\text{Recovery}_{\text{in vivo}} = 1 - \frac{\text{Cout}}{\text{Cin}} = 1 - \frac{\text{PA}_{\text{UK-54,373}}(\text{out})}{\text{PA}_{\text{UK-54,373}}(\text{in})} \quad (3)$$

where $\text{PA}_{\text{UK-54,373}}$(out) was the peak area of UK-54,373 in the dialysate leaving the probe, and $\text{PA}_{\text{UK-54,373}}$(in) was the peak area of UK-54,373 in the perfusate entering the probe. The concentration of fluconazole in the dialysates from the brain extracellular fluid was then corrected using the *in vivo* recovery to obtain the fluconazole concentration-time profile for the cortical extracellular fluid.

The plasma fluconazole concentration-time profiles following fluconazole IV bolus dosing were characterized by a monoexponential decline. The elimination half-lives of fluconazole in plasma and brain were calculated from the slopes of the fluconazole logarithmic concentration-time plots. The fluconazole area under the plasma concentration-time curve (AUCp) was corrected for free fraction and calculated using linear trapezoidal rule and extrapolated to infinite time by the addition of $\text{Cp}_{\text{last}}/\lambda n$, where Cp_{last} was the concentration at the last measurement time and λn was the terminal elimination rate constant. The area under brain concentration-time curve was calculated as the sum of the products of the measured concentration in an interval and the time of the collection interval, with addition of the residual area to obtain the AUC to infinite time. This can be done because the concentration measured in the dialysate is essentially the average concentration over the collection interval and as such, an integrated response value (the time averaged concentration). Therefore, the AUCbrain can be calculated from the following equation:

$$\text{AUC}_{\text{brain}} = \sum_{i=1}^{n} Ci\Delta t + \frac{Cn}{\lambda n} \quad (4)$$

where Cn is the concentration at the last measurement time and λn is the terminal elimination rate constant.

The clearance ratio, Clin/Clout, describes the transfer between the tissue and the plasma. It reflects the distribution of drug between the plasma and the specific brain region and is the equilibrium distribution coefficient or "partition coefficient" at steady state and therefore describes the affinity of the tissue for the drug. This ratio for a specific tissue can be calculated from the ratio of areas under the curve as follows:

$$\frac{\text{Clin}}{\text{Clout}} = \frac{\text{AUC}_{\text{tissue}}}{\text{AUC}_{\text{plasma}}} \quad (5)$$

where $\text{AUC}_{\text{tissue}}$ is the area under the brain ECF fluconazole concentration-time curve to infinite time, $\text{AUC}_{\text{plasma}}$ is the area under the plasma fluconazole concentration-time curve to infinite time, Clin is the distributional clearance from the plasma to brain, and Clout is the distributional clearance from the brain to plasma.

Statistical moment analysis was used to obtain the total plasma clearance of fluconazole (Cltot = dose/AUCp), and the volume of distribution at steady state (Vdss = dose*AUMC/AUC^2), and mean residence time (MRT = AUMC/AUC), where the AUMC is the area under the first moment curve.

RESULTS

Recovery of Fluconazole and UK-54,373 *In Vitro*

We compared the recovery by gain (RG) and by loss (RL) for fluconazole and UK-54,373 *in vitro* for the preliminary validation of UK-54,373 as the retrodialysis calibrator. There was no statistical difference between the RG and RL for either compound (Table I). The RG of fluconazole was not statistically different from the RL of UK-54,373. These results indicate that fluconazole and UK-54,373 have similar dialysis characteristics *in vitro*, and that UK-54,373 may be used as the probe calibrator

Table I. Comparison of the Relative Recovery Between Fluconazole and UK-54, 373 *In Vitro*[a]

Probe	%RG[b] (UK-54, 373)	%RG[b] (fluconazole)	Ratio (UK/Flu)
1 (n = 8)	73.2 ± 4.6	71.9 ± 3.4	98.3 ± 2.1
2 (n = 10)	71.4 ± 5.9	70.8 ± 5.8	95.0 ± 2.5
Average ± S.D.	72.4 ± 1.2	71.7 ± 1.1	96.7 ± 2.3
Probe	%RL[c] (UK-54, 373)	%RL[c] (fluconazole)	Ratio (UK/Flu)
1 (n = 20)	71.5 ± 1.6	72.4 ± 2.0	101.2 ± 1.1
2 (n = 20)	65.5 ± 1.6	69.0 ± 1.6	105.3 ± 0.8
Average ± S.D.	68.5 ± 4.2	70.7 ± 2.5	103.3 ± 2.9

[a] CMA-12 3 mm microdialysis probes, perfusion rate: 0.5 μl/min, 37°C.
[b] Recovery by gain.
[c] Recovery by loss.

Table II. Comparison of the Recovery by Loss of Fluconazole Versus UK-54, 373 *In Vivo* in Rat Brain Tissues[a]

Rat	%RL[b] (fluconazole)	%RL[b] (UK-54, 373)	Ratio (flu/UK)
1 (n = 6)[c]	12.7 ± 1.4	13.7 ± 3.7	93.0
2 (n = 5)	15.9 ± 1.2	13.6 ± 1.1	116.7
3 (n = 5)	21.0 ± 3.8	23.9 ± 2.7	88.1
4 (n = 6)	37.0 ± 12.8	38.2 ± 12.5	97.0
Average ± S.D.	21.6 ± 10.8	22.4 ± 11.6	98.7 ± 12.5

[a] CMA-12 3 mm microdialysis probes, perfusion rate 0.5 μl/min.
[b] Recovery by loss.
[c] number of collection periods.

for estimating the recovery of fluconazole. Furthermore, the recovery has no directional dependence suggesting that the retrodialysis (recovery by loss) can be directly used to estimate the recovery from the medium surrounding the probe.

Recovery by Loss (RL) of Fluconazole and UK-54,373 in Brain

The RL for fluconazole and UK-54,373 was compared *in vivo* in the rat brain tissue to further validate the feasibility of using UK-54,373 as the probe calibrator. The RL of fluconazole (21.6% ± 10.8%) was not significantly different from that of UK-54,373 (22.4% ± 11.6%), by paired t-test (see Table II). Moreover, the RL of fluconazole and UK-54,373 in brain tissue was significantly less than the RL of the same compounds when the probe is placed in artificial CSF at the same perfusion rate. These results suggest that *in vitro* probe calibration is not appropriate to estimate the probe recovery *in vivo* in the brain tissue. It is necessary to determine the probe recovery in the tissue of interest, and the retrodialysis of UK-54,373 can be used to estimate the probe recovery in brain tissue for fluconazole.

Fluconazole Brain Distribution Studies

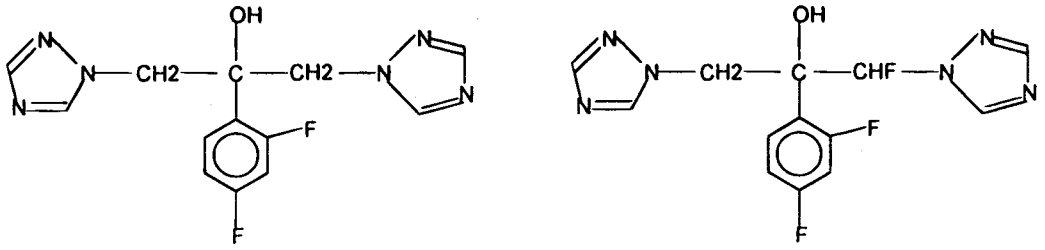
Fluconazole Plasma Pharmacokinetics

The free fraction of fluconazole in rat plasma was 0.92 ± 0.04, as determined by ultrafiltration. This value is similar to previous reports of fluconazole free fraction in rat plasma, 0.91 (16) and 0.88 (17).

The fluconazole plasma pharmacokinetic parameters, corrected for free fraction and determined following intravenous bolus dosing, are listed in Table III. There was no statistical difference (by paired t-test, $p < 0.05$) in these parameters between 10 mg/kg and 20 mg/kg doses, and no period effects were seen in this crossover design.

Fluconazole Brain Distribution

Fluconazole rapidly reached a distributional equilibrium between brain ECF and free plasma concentration (Fig 3). The distribution to the brain ECF was substantial, with the equilibrium distribution coefficients ranging from 0.37 to 0.84 (Table IV). The extent of the distribution of fluconazole to the brain was proportional to the dose over the two doses studied, i.e., there was no statistical difference between the distribution coefficients observed following the two doses. Terminal phase half-lives were not different between the plasma (367.8 ± 47.7 min) and the brain (380.0 ± 107.6 min). No treatment order effect was seen in the plasma pharmacokinetic parameters and the brain distribution coefficients, indicating a stable preparation over the course of the crossover study. The relative recovery of the brain microdialysis probe (as measured *in vivo* by retrodialysis of UK-



Fluconazole **UK-54373**

Fig. 1. Chemical structures of fluconazole and UK-54,373.

Table III. Fluconazole Plasma Pharmacokinetic Parameters

Dose (mg/kg)	Cl[a] (ml/min/kg)	Vdss[b] (L/kg)	MRT[c] (min)	AUC[d] (μg*min/L)
10 (n = 6)	2.42 ± 0.74	1.18 ± 0.32	490.3 ± 69.5	4456 ± 1275
20 (n = 6)	2.43 ± 0.50	1.17 ± 0.21	473.3 ± 71.4	8522 ± 1670
Average	2.42 ± 0.60	1.17 ± 0.26	481.8 ± 72.1	

[a] Total plasma clearance.
[b] Volume of distribution at steady state.
[c] Mean residence time in plasma.
[d] Area under the plasma concentration-time curve.

Table IV. Fluconazole Brain Distribution Kinetics

Dose (mg/kg)	$T1/2_{brain}$ (min)	$T1/2_{plasma}$ (min)	AUC_{brain} (μg*min/L)	AUC_{brain}/AUC_{plasma}
10 (n = 6)	423 ± 126	390 ± 45	2590 ± 429	0.62 ± 0.20
20 (n = 6)	338 ± 72	346 ± 43	4825 ± 1692	0.57 ± 0.16
Average	380 ± 108	368 ± 48		0.60 ± 0.18

54,373) was approximately 20–25% at the flow rate of 0.5 μl/min, and did not exhibit time-dependent trends (Fig 2).

DISCUSSION

When using microdialysis as a sampling tool in pharmacokinetic and drug distribution studies, the accurate determination of drug concentration in the plasma or tissues surrounding the probe is dependent upon knowing the *in vivo* relative recovery of the probe. Typically, the *in vitro* recovery cannot estimate *in vivo* recovery (18,19), because the probe efficiency may change in the *in vivo* condition due to the possible interaction of the plasma or tissue components with the membrane materials, and because the diffusivity of molecules through the tissue *in vivo* is different from the diffusivity *in vitro* and diffusion through the tissue may be the primary determinant of probe recovery (20). It was observed in the present study that the *in vitro* recovery by loss of fluconazole and UK-54,373 was significantly greater than the *in vivo* recovery from probes placed in the brain. This result indicates that *in vivo* probe calibration is necessary for the accurate estimation of tissue drug concentration.

The *in vitro* recovery experiments showed that the recovery by loss and recovery by gain of fluconazole was identical to that of UK-54,373, and there was no directional difference in the recovery for both fluconazole and UK-54,373. Furthermore, fluconazole has the identical recovery by loss in brain tissue as UK-54,373. These results suggest that fluconazole and UK-54,373 have the same probe efficiency and diffusion resistance in brain tissue, therefore retrodialysis with UK-54,373 yields continuous recovery values that can be used to calculate the brain ECF fluconazole concentration.

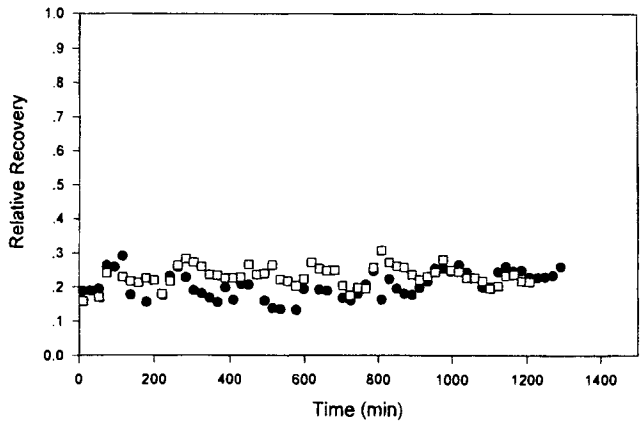
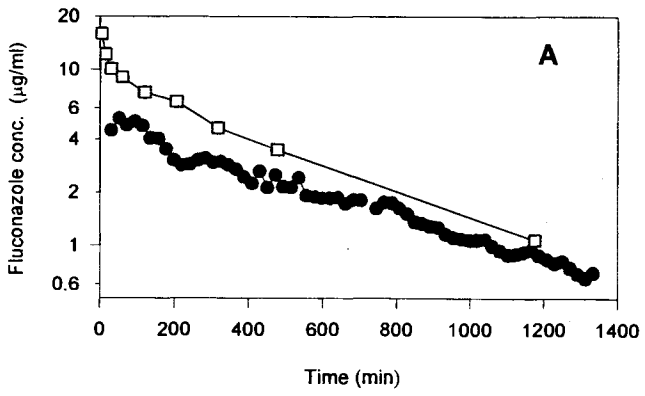


Fig. 2. Relative probe recovery in vivo (retrodialysis of UK-54,373) for rat #3. (●) phase one of crossover, (□) phase two of crossover.

An important consideration in the use of intracerebral microdialysis for drug distribution studies is the integrity of the blood-brain barrier following probe placement. Recently there have been a few studies questioning the healing of the blood-brain barrier after probe insertion (21,22). In another study from our laboratory, we measured the cortex ECF to plasma AUC ratios (corrected for in vivo probe recovery) for ^3H_2O and ^{14}C-mannitol 18 hours after probe placement in the freely-moving rat. Following intravenous injection of each marker, the relative penetration (AUCcortex/AUCplasma) of water was 103% (n = 2) and the penetration of mannitol was 9.3% (n = 6), indicating the chemical selectivity of the BBB permeability was intact in our preparation (23).

The plasma pharmacokinetic parameters obtained in this study (table III) are comparable with previously reported data



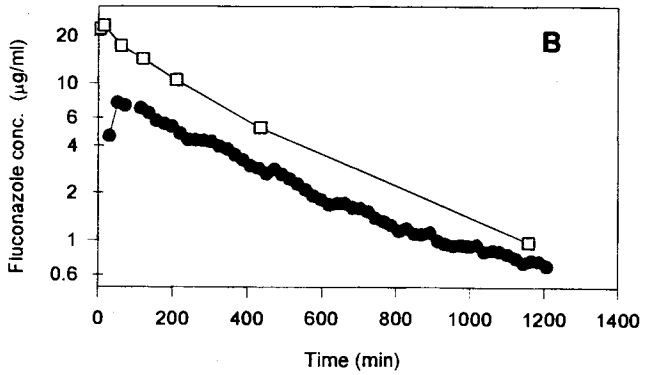


Fig. 3. Fluconazole brain distribution study in rat #3, following 10 mg/kg IV bolus dose in phase one of crossover (a), and following 20 mg/kg IV bolus dose in phase two of crossover (b). (●) fluconazole concentration in cortical extracellular fluid; (□) fluconazole concentration in plasma.

(17). In a study where a 20 mg/kg oral dose of fluconazole was administered to female CD rats, the fluconazole volume of distribution was 0.8 L/kg, the elimination half-life was approximately 4 hours, and the total plasma clearance was 2.2 ml/min/kg (17). In the present study, fluconazole exhibits linear plasma pharmacokinetics in the 10 to 20 mg/kg dose range, which is in agreement with the reported results in mice (17).

There are several reports about the distribution of fluconazole into CSF in humans and animals (24,25). However, there is only one report on the distribution of fluconazole into brain tissue (26), which determined the fluconazole concentration in healthy brain tissue homogenates in patients (n = 4) who had a deep cerebral tumor whose removal required the excision of healthy brain tissue. This study found the average concentration ratio of brain homogenate to plasma was 1.33 at steady state (range, 0.7 to 2.39). It is difficult to draw a conclusion from this experiment, considering these patients were taking multiple medications and the fluconazole concentration ratios were determined at a single time point after dose administration. Our results indicate that fluconazole can rapidly penetrate into the brain ECF, with the brain ECF peak fluconazole concentration achieved within 60 minutes following IV bolus doses. The distribution equilibrium is rapidly achieved with an average brain ECF to plasma distribution coefficient of 0.60. The brain distributional kinetics and brain to plasma distribution coefficient obtained from our study is similar to the reported CSF to plasma distribution coefficient in humans and animals (1,24,25), which suggests that fluconazole concentration in the brain ECF may be similar to fluconazole concentration in CSF. Characterizing the relationship between fluconazole concentration in brain ECF and in CSF is of clinical importance, since fluconazole concentration in CSF is used to estimate the penetration of the drug into the CNS, and the actual site of infection is in the brain tissue. Additional microdialysis experiments are underway to address this issue.

In this study, the intracerebral microdialysis technique, using a simultaneous *in vivo* retrodialysis calibrator, has been validated to determine brain fluconazole concentrations and CNS distributional parameters. 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 and the distribution of fluconazole to the cortical ECF was linearly related to dose. This finding indicates that a clinician may expect a proportional increase in brain exposure of the drug with an increase in dose. Future applications of this model may include antifungal transport studies in a model of fungal meningitis with fluconazole and other antifungals designed to penetrate the blood-brain barrier.

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