Fluconazole Penetration in Cerebral Parenchyma in Humans at Steady State

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We studied fluconazole penetration in the brain in five patients who had a deep cerebral tumor whose removal required the excision of healthy brain tissue. Plasma and brain samples were simultaneously obtained after oral ingestion of 400 mg of fluconazole daily for 4 days (90% of steady state). Fluconazole penetration in healthy cerebral parenchyma was determined. Plasma and brain samples were assayed by high-pressure liquid chromatography. Concentrations in plasma and brain tissue were 13.5 ± 5.5 μg/ml and 17.6 ± 6.6 μg/g, respectively. The average ratio of concentrations in the brain and plasma (four patients) was 1.33 (range, 0.70 to 2.39). Despite the lack of data concerning the penetration of fluconazole in brain abscesses, these results should permit the use of a daily dose of 400 mg of fluconazole in prospective clinical studies that evaluate the effectiveness of this drug in the treatment of brain abscesses due to susceptible species of fungi.

Fluconazole, a triazole compound, is a systemic antifungal synthetic agent. Its fungistatic action is the inhibition of fungal cytochrome P-450 sterol C-14 alpha demethylation (18). During recent years, an increase in the rate of central nervous system fungal infections mainly due to an increased prevalence of immunosuppressed states (e.g., AIDS, malignancies, and organ transplantation) has been reported. Treatment by fluconazole of invasive infections due to various fungi has been a significant advance in antimicrobial chemotherapy (1, 19, 21). Its efficacy in treating cryptococcal and coccidioidal meningitis (2, 3, 5, 14, 22, 23) is well established in clinical studies.

In acute AIDS-associated cryptococcal meningitis fluconazole was shown to be as effective as amphotericin B (23) and more so in preventing relapse (3, 22). However, use of fluconazole for treatment of this disease remains controversial (6, 20, 24), and the optimal dose remains uncertain. The incidence of cryptococcal intracerebral lesions is unknown and probably underestimated (6, 8, 11, 27). Inadequate control of these lesions is perhaps one of the risk factors for failure or relapse of cryptococcal meningitis. Actually, therapeutic failures and relapses may occur when using amphotericin B or fluconazole (400 mg/day), and higher doses of fluconazole as salvage therapy have been proposed (2).

While the penetration of fluconazole in human cerebrospinal fluid is known to be good (60 to 80% of levels in serum) (10, 25), data regarding its penetration in brain tissue other than in animals are lacking (17, 26). The objective of this research was to study the penetration of fluconazole in human brain tissue and to determine the ratio of concentrations in the brain and plasma at steady state.

MATERIALS AND METHODS

Obtaining brain tissue samples from patients with cryptococcal meningitis is not acceptable in light of current diagnostic and therapeutic strategies. Obtaining tissue samples from healthy volunteers is not conceivable. We therefore decided to study the penetration of fluconazole in the healthy cerebral parenchyma of selected patients presenting with a deep-seated tumor, for whom neurosurgical procedure required the excision of healthy brain tissue, which is usually discarded.

This protocol, which included ingestion of a drug (fluconazole) unnecessary to treatment but of little toxicity, was approved by the ethics committee of the Société de Réanimation de Langue Française. All subjects gave their informed consent prior to their participation in the study.

Patients. Among adult patients presenting with a deep tumor whose excision would also require that of healthy brain tissue, the following groups were excluded from participation in the study: pregnant or fertile women without contraception, patients who had a plasma creatinine level of 30% above normal, patients treated previously with fluconazole, and patients who had not given their informed consent.

Prior to enrollment, each subject had a complete medical history taken and a physical examination, and a blood count and blood chemistries (creatinine, alkaline phosphatase, aspartate transaminase, alanine transaminase, and bilirubin) were done. Patients took orally 400 mg of fluconazole at 8 a.m. each day for 4 days. Blood count and blood chemistries were repeated on the fourth day. For the prevention of seizures, 200 mg of phenobarbital was given the morning of the surgery. Surgical removal of the tumor was performed 4 h after the last ingestion of fluconazole.

Fluconazole assay. While performing the excision of healthy brain tissue, a blood sample was obtained. To control for contamination with blood, the brain sample was rinsed with saline isotonic solution and dried with a gauze. The plasma and brain samples were frozen at −70°C until assayed for fluconazole. After defrosting, the entire brain sample was weighed and homogenized.

Plasma and brain samples were assayed for fluconazole by high-pressure liquid chromatography according to the method recommended by Pfizer Central Research, Sandwich, Kent, United Kingdom. Briefly, the samples were made basic with 1 M sodium hydroxide (1 ml) and extracted with ethyl acetate (2 ml). The organic phase was back extracted with 1 M HCl (2 ml) and then discarded. The aqueous phase was again made basic with 5 M NaOH (1 ml); this step was followed by extraction with ethyl acetate (4 ml) and then evaporation to dryness. The samples were reconstituted with 10 μl of mobile phase (0.2 M TEMED [N,N,N’,N’-tetramethylethylenediamine] [pH 7.0] and acetonitrile). Detection was achieved by UV spectrophotometry. The limit of quantification was 0.15 μg/ml. Interrun coefficients of variation of the method were 12% for 0.25 μg/ml and 5% for 2 μg/ml. The range of assay linearity was 0.25 to 20 μg/ml.

RESULTS

Six patients with a diagnosis of deep intracerebral tumor were enrolled. The tumor was a meningioma in all cases. The characteristics of the patients are described in Table 1. The ingested dose per day was 6.5 ± 1 mg/kg of body weight. All patients absorbed fluconazole until the time of surgical procedure. Performing an excision of healthy brain tissue was found unnecessary in one patient (patient no. 4) presenting with a deep cerebellum meningioma. Only five patients (mean age, 41.8 ± 16.9 years) were thus evaluated. For one patient (no. 2)
assays could be done only in the brain sample. The ratio of concentrations in the brain and plasma was assessed in only four patients.

At the fourth day of treatment with the 400-mg daily dose, 4 to 5 h after the last ingestion, the plasma fluconazole concentration was $13.5 \pm 3.5 \mu g/ml$ (range, 10.4 to 18.2 $\mu g/ml$) and the brain fluconazole concentration was $17.6 \pm 6.6 \mu g/g$ (range, 10.2 to 27 $\mu g/g$). The ratio of concentrations in the brain and plasma, done only for the four patients who had concentrations in both serum and brain tissue measured, was $1.33 \pm 0.74$ (range, 0.70 to 2.39). The results are shown in Table 2.

No serious adverse effect was observed other than an asymptomatic increase in plasma aminotransferase (1.5 times the normal value) in one patient (no. 5) which was reversible 48 h after fluconazole administration was stopped.

**DISCUSSION**

The use of fluconazole in meningeal or cerebral fungal diseases has remained controversial (20) until recently. Its use may be justified by its lower toxicity in comparison to amphotericin B and by a better knowledge of the appropriate regimen. When fluconazole is used, a daily dose of 400 mg is at present the standard dosage in the treatment of both systemic candidiasis and cryptococcal meningitis. A recent study indicates that fluconazole (200 to 400 mg daily) is an effective and better-tolerated alternative to amphotericin B in acute AIDS-associated cryptococcal meningitis (23). Higher doses have been proposed in case of failure of treatment with amphotericin B or with the standard regimen of fluconazole (2). However, the choice of the optimal dosage should be based on data concerning drug concentrations at the site of infection in specific tissues, such as in the brain. These data would best be obtained at steady state, so that the extrapolation to clinical situations may be facilitated.

The pharmacologic properties of fluconazole in humans are well known: oral bioavailability is 80%, protein binding is 11%, and apparent volume of distribution ranges from 0.62 to 0.82 liter/kg. The drug is eliminated mostly (80%) by the kidney in unchanged form, and the half-life varies from 22 to 31 h (4, 7, 15, 17). The time of peak level after oral ingestion of fluconazole is 2 to 4 h. The peak concentration in plasma is proportional to the dose, and after a single oral dose of 400 mg the maximum concentration of fluconazole in plasma is 6.7 $\mu g/ml$ (15). At steady state, the peak concentration in plasma is approximately 2 to 2.5 times higher than after a single dose. The steady state is obtained after 6 to 10 days, but 90% of the value at the steady state is obtained on the fourth to fifth day (4). In this study, in which fluconazole was not required for the patients’ treatment, the administration of a standard dose of fluconazole (400 mg) for 4 days was thought to be a good compromise between the necessities of sampling at steady state and of adherence to ethical requirements.

Concentrations of fluconazole in body fluids have been previously reported (10, 25); however, detailed studies of concentrations in tissues are lacking. Apart from two animal studies (17, 26), only one study of humans provides data on fluconazole concentrations in the brain (9). In that study the distribution of fluconazole was determined by positron emission tomographic scanning over a 2-h period following a single infusion of 400 mg of fluconazole with a tracer of $[18F]$fluconazole. The positron emission tomography images showed that fluconazole distribution in the brain was very uniform, with concentrations varying from 4.20 $\pm 0.4 \mu g/g$ in the occipital cortex to 5.48 $\pm 0.25 \mu g/g$ in the cerebellum, when the concentration in blood was 3.76 $\mu g/ml$. However, these results were obtained after a single infusion, whereas relevant concentrations are those obtained in clinical situations at steady state, following repeated administrations.

In our study, at 90% of the steady-state value, the mean concentration in plasma 4 h after the last ingestion was $13.5 \pm 3.5 \mu g/ml$, which is in agreement with the known pharmacokinetic properties of fluconazole. At that time, the mean concentration in the brain was $17.6 \pm 6.6 \mu g/g$. In keeping with the study cited above, the brain-to-plasma ratio was slightly higher than 1. While a definite answer ideally requires serial sampling of brain tissue, which is, alas, impossible, fluconazole can nevertheless be considered to have a good penetration in brain tissue, with levels in the brain closely paralleling those in plasma.

**TABLE 1. Characteristics of patients**

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (yrs)</th>
<th>Sex</th>
<th>Localization of tumor</th>
<th>Weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>65</td>
<td>M</td>
<td>Frontal cortex</td>
<td>76.9</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>F</td>
<td>Cerebellum</td>
<td>57.1</td>
</tr>
<tr>
<td>3</td>
<td>51</td>
<td>F</td>
<td>Parietal cortex</td>
<td>54.8</td>
</tr>
<tr>
<td>4</td>
<td>45</td>
<td>M</td>
<td>Cerebellum</td>
<td>68.2</td>
</tr>
<tr>
<td>5</td>
<td>26</td>
<td>F</td>
<td>Cerebellum</td>
<td>71.4</td>
</tr>
<tr>
<td>6</td>
<td>42</td>
<td>F</td>
<td>Lateral ventricle</td>
<td>55</td>
</tr>
</tbody>
</table>

* M, male; F, female.

* All tumors were meningiomas.

* Not evaluated (excision of healthy brain tissue was found to be unnecessary).

**TABLE 2. Results**

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Plasma fluconazole concn ((\mu g/ml))</th>
<th>Brain fluconazole concn ((\mu g/g))</th>
<th>Brain/plasma ratio</th>
<th>Fluconazole dose (mg/kg)</th>
<th>Sampling delay after last ingested dose (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10.4</td>
<td>10.2</td>
<td>0.99</td>
<td>5.2</td>
<td>240</td>
</tr>
<tr>
<td>2</td>
<td>ND(^b)</td>
<td>20.5</td>
<td></td>
<td>7</td>
<td>240</td>
</tr>
<tr>
<td>3</td>
<td>18.2</td>
<td>12.8</td>
<td>0.70</td>
<td>7.3</td>
<td>240</td>
</tr>
<tr>
<td>5</td>
<td>14.2</td>
<td>17.7</td>
<td>1.25</td>
<td>5.6</td>
<td>300</td>
</tr>
<tr>
<td>6</td>
<td>11.3</td>
<td>27.0</td>
<td>2.39</td>
<td>7.3</td>
<td>270</td>
</tr>
</tbody>
</table>

Average $\pm$ SD\(^c\)

| 13.5 $\pm$ 3.5 | 17.6 $\pm$ 6.6 | 1.33 $\pm$ 0.74 | 6.5 $\pm$ 1.0 | 258 $\pm$ 27 |

\(^a\) Only four patients were evaluated.

\(^b\) ND, not determined.

\(^c\) SD, standard deviation.
Fluconazole is active against *Candida neoformans* (MIC, 1.25 to 6.25 μg/ml), *Candida albicans* (MIC, 0.125 to 0.8 μg/ml), *Candida krusei* (MIC, 0.19 μg/ml), *Candida guilliermondii* (MIC, 0.62 μg/ml), and *Candida parapsilosis* (MIC, 0.39 to 3.13 μg/ml); less active against *Candida tropicalis* (MIC, 0.78 to 6.25 μg/ml) and *Candida glabrata* (MIC, 1.9 to 12.25 μg/ml); and inactive against *Candida kruizi* (MIC > 25 μg/ml) and *Aspergillus* species (MIC > 100 μg/ml) (15, 16). Thus, according to the fluconazole concentration values obtained in brain samples at steady state in serum, treatment with fluconazole of brain abscesses due to susceptible fungal species appears possible. However, the activity of fluconazole measured in vitro against selected yeasts may vary significantly, depending on assay methods. The activity of an azole antifungal drug can be affected by the culture medium and its pH, the inoculum size, and the incubation temperature or duration (12). Because of a lack of standardized tests, the comparisons of MICs from laboratory to laboratory and from study to study should be interpreted with caution. Moreover, no relationship has been established between in vitro results and patients’ responses (13).

**Conclusion.** The concentration of fluconazole obtained at steady state in healthy brain parenchyma was shown in this study to be much greater than the MICs for susceptible yeasts. Therefore, in spite of the problems linked to the interpretation of assays performed in vitro and of the lack of data concerning penetration of fluconazole in brain abscesses, its use in the treatment of brain abscesses due to susceptible fungi is an attractive alternative in that this drug has little toxicity and a prolonged half-life. Indeed, the good penetration of fluconazole in brain tissue and the possibility of indirect monitoring of values in plasma according to the observed brain-to-plasma ratio would permit clinical studies of this drug, which are required to confirm its effectiveness in the treatment of brain infections due to susceptible fungi other than cryptococcal meningitis. These studies would be facilitated if an accepted and standardized method of susceptibility testing of fungi were available.

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**REFERENCES**


