Comparative Efficacies of Terbinafine and Fluconazole in Treatment of Experimental Coccidioidal Meningitis in a Rabbit Model

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A rabbit model of coccidioidal meningitis was used to compare the therapeutic efficacies of terbinafine (TBF) and fluconazole (FCZ). Hydrocortisone acetate-treated New Zealand White male rabbits were infected intracisternally with either $2.2 \times 10^4$ or $6.4 \times 10^3$ Coccidioides immitis arthroconidia. Oral treatment with polyethylene glycol 200 (PEG) twice daily ($n = 8$), TBF twice daily ($n = 9; 200$ mg/kg of body weight/day), or FCZ once daily ($n = 8; 80$ mg/kg/day) began on day 5 and continued for 21 days. Mean survival times were 20, 24, and 32 days for rabbits treated with PEG, TBF, and FCZ, respectively. All of the FCZ-treated animals ($100\%$; $P = 0.003$), 56% of the TBF-treated animals ($P = 0.4$), and 25% of the PEG-treated animals survived the length of the study. Both FCZ and TBF were effective at reducing the incidence of paresis. Only FCZ was effective at reducing most neurological and systemic signs. FCZ treatments resulted in lower cerebrospinal fluid (CSF) protein concentrations and leukocyte counts and faster clearing of CSF fungal cultures compared with those for PEG-treated controls, but TBF treatments had no significant effect on these parameters. Neither drug affected CSF glucose levels. Mean serum TBF levels by bioassay were within the range of $3.5$ to $6.2 \mu$g/ml at $1, 2,$ and $4 \text{ h}$ postdosing and $0.35$ to $7.0 \mu$g/ml at $14 \text{ h}$ postdosing. No TBF was detected in CSF. Mean FCZ levels ($24$ to $25.5 \text{ h}$ postdosing) by bioassay were $16.4$ to $19.2$ and $13.5$ to $19.2 \mu$g/ml in serum and CSF, respectively. The reduction in the numbers of CFU in the spinal cord and brain was over $100\times$ ($P = 0.0005$) in FCZ-treated animals and $2 \times$ ($P = 0.2$) in TBF-treated animals compared with those in PEG-treated animals. Histopathologic severity (semi-quantitative scoring system) was significantly attenuated by FCZ treatment ($P = 0.05$) and was slightly attenuated by TBF treatment compared with that for the controls. In conclusion, TBF appeared to have a slight effect on survival, histology, and reduction of the numbers of CFU in tissue; however, these effects were not significant. FCZ was effective at controlling coccidioidal meningitis.

Coccidioidal meningitis is one of the most severe and devastating fungal diseases. Currently, therapy with an azole will control this meningitis in $80\%$ of patients. This therapy is usually considered lifelong, and a cure is unusual (2). Patients receiving azole therapy are still at risk for other complications associated with coccidioidal meningitis, such as stroke, vasculitis, hydrocephalus, and encephalitis (17).

Terbinafine (TBF) is a newly developed agent with a mechanism of action distinct among antifungal drugs. TBF inhibits fungal growth by inhibiting the fungal enzyme squalene epoxidase, a key enzyme in the synthesis of the fungal sterol ergosterol (1). TBF is fungicidal in vitro against a broad range of fungi (9). The drug is highly lipophilic and distributes widely into adipose and keratin-rich tissues (1, 6). Tissue TBF concentrations are much higher than plasma TBF concentrations, and it is highly bound to plasma components (11). This protein binding along with lipoprotein binding and rapid drug metabolism by rodents has been suggested as the reason for the lack of efficacy of TBF in experimental models of systemic fungal infection. However, a few reports have suggested that TBF may be effective for the treatment of systemic fungal infections (12, 16).

This study evaluated the efficacy of TBF in a rabbit model of coccidioidal meningitis in comparison with that of a conventional treatment, fluconazole (FCZ).

MATERIALS AND METHODS

Animals and study design. Male New Zealand White rabbits (weight, 3 to 4 kg) were used in the study. To avoid potential complications with opportunistic pathogens in the rabbit, these rabbits were specific pathogen free and were free of Bordetella. The study was done in two parts. Each part used 12 to 13 rabbits, with 4 or 5 rabbits per treatment group.

Immune suppression. At $1 \text{ day}$ prior to infection, on the day of infection, and for $3 \text{ consecutive days}$ following infection, all rabbits received an intramuscular injection of hydrocortisone acetate (Steris Laboratory, Inc., Subsidiary of Schein Pharmaceutical, Inc., Florham Park, N.J.) at $2 \text{ mg/kg}$ of body weight.

Test organism. Coccidioides immitis strain Silveira (ATCC 28868) was used in the study with appropriate biohazard containment. A suspension of arthroconidia of C. immitis was prepared as described previously (18). The suspension was stored at $4°C$.

Susceptibility testing. Sensitivity testing in vitro was performed as described previously for arthroconidia (14) and endospores (5).

Infection. Prior to infection, the stock suspension of arthroconidia was quantitated by serial plating on Mycosel agar plates (Becton Dickinson and Co., Cockeysville, Md.). The stock suspension was further diluted to make an inoculum containing $2.5 \times 10^6$ CFU/ml. Each rabbit was anesthetized and infected intracisternally as described previously (18). Rabbits were infected with either $2.2 \times 10^4$ or $6.4 \times 10^4$ arthroconidia in parts 1 and 2, respectively. Up to 0.6

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ml of cerebrospinal fluid (CSF) was removed by gentle aspiration, and then 0.2 ml of phosphate-buffered saline and plated on Mycosel agar. The detectable level was less than 4 CFU/ml of CSF.

Collection of CSF and serum. Every 7 to 12 days, rabbits were anesthetized to a surgical plane of anesthesia with isoflurane, and CSF and serum were collected as described previously (18).

Euthanasia and tissue collection. Rabbits that required euthanasia or that survived 7 or 8 days after the last treatment (32 or 33 days postinfection) were anesthetized, CSF and serum were collected, and the rabbits were euthanized by intravenous injection of a concentrated pentobarbital solution (Euthanol; Delmarvia Laboratories, Inc., Bristol, Ten.). After euthanasia, the brains and proximal spinal cords were collected. The left half of the brain including the cerebellum and upper cervical cord was transected and placed into 10% buffered formalin for histopathologic study. The right half of the specimen was transected into the brain (cerebrum, cerebellum, pons, and medulla) and proximal spinal cord (approximately 1.5 cm) and was processed separately for quantitative fungal culture as described previously (18).

Histopathology scoring. The central nervous system was sectioned in a paraffin block containing the cerebrum, brain stem, midbrain, cerebellum, and upper cervical cord sections. Sections were stained with hematoxylin-eosin and periodic acid-Schiff. The grade by 5 in: (i) placebo (in saline at 200 [PEG] mg/kg), daily (n = 8); or (ii) TBF at 100 mg/kg twice daily (n = 9) starting 5 days after infection.

RESULTS

In vitro susceptibility. The activities of TBF and FCZ against C. immitis strain Silveira were tested in vitro by the broth macrodilution method. TBF and FCZ MICs for the mycelial form were 0.3 and 6.25 µg/ml, respectively. The TBF MIC for the parasitic form (endospores) of the fungus was 0.6 µg/ml.

Survival. Overall, two of eight PEG-treated rabbits survived to the end of the study, whereas five of nine (P = 0.2 by the Fisher exact test) and eight of eight (P = 0.004) TBF- and FCZ-treated rabbits survived, respectively. One designated additional TBF-treated animal was used for drug and tissue studies only and was not included in the survival study. Survival was not significantly affected by TBF treatment, and there was only a suggestion of efficacy. FCZ treatment was borderline superior compared to TBF treatment (P = 0.053).

When time to death was analyzed by the Kaplan-Meier test (Fig. 1), treatment with FCZ prolonged survival over the survival times after both PEG and TBF treatments (P ≤ 0.04). TBF treatment did not prolong survival compared to the survival time after the control PEG treatment (P = 0.4). Mean survival times were 20, 32, and 24 days for PEG-, FCZ-, and TBF-treated animals, respectively.

Fungal tissue burden. The same nonsignificant suggestion of efficacy seen from the survival of TBF-treated animals was also seen in the reduction of C. immitis counts in the spinal cords and brains (Fig. 2). A twofold reduction in the numbers of CFU of C. immitis in both the spinal cords and brains of TBF-treated animals was seen compared to the numbers in the PEG-treated animals (P ≤ 0.19). FCZ-treated animals showed over a 100-fold reduction in the numbers of CFU in both tissues compared to the numbers in both tissues of PEG-treated animals (P ≤ 0.0005). FCZ treatment was better than TBF treatment (P ≤ 0.0004). None of the tissues from any treatment group were free of detectable infection after 3 weeks of treatment.
mals ($P < 0.05$) but not TBF-treated animals. Endarteritis was noted in four of eight PEG-treated animals and in four of eight and seven of nine animals treated with FCZ and TBF, respectively; ischemia and/or infarcts were noted in seven, six, and five animals treated with PEG, TBF, and FCZ, respectively.

**CSF WBC concentrations.** Before infection, rabbits had few WBCs in their CSF ($0, 16 \pm 44$, and $28 \pm 55$ WBCs/mm$^3$ in the PEG-, FCZ-, and TBF-treated groups, respectively). Only three animals in all the groups had any WBCs in their CSF. This was most likely related to the trauma of initial puncture. After infection, mean WBC counts increased dramatically (range, 1,297 to 2,522 WBCs/mm$^3$) and did not return to baseline (day 0) levels for any of the treatment groups ($P \leq 0.05$) throughout the study (Fig. 3A). WBC counts for TBF-treated animals were not different from those for PEG-treated animals throughout the study. FCZ caused a reduction in WBC counts on days 25 to 26 and 32 to 33 compared to those for PEG-treated ($P = 0.01$) and TBF-treated ($P = 0.02$) animals sampled on the same days. FCZ treatment reduced WBC counts on days 25 to 26 and 32 to 33 compared to those on days 11 to 12 ($P = 0.005$) and 32 to 33 compared to those on days 18 to 19 ($P = 0.008$).

**CSF glucose concentrations.** CSF glucose concentrations were similar for all treatment groups throughout the study. Glucose concentrations decreased from $104.1 \pm 12.7$, $96.6 \pm 9.9$, and $103.6 \pm 9.2$ mg/dl before infection to $58.5 \pm 15.9$, $51.0 \pm 6.7$, and $50.3 \pm 4.8$ mg/dl for PEG-, FCZ-, and TBF-treated animals, respectively, 11 to 12 days after infection with *C. immitis*. This decline was followed by a slow rebound for all groups and was not dependent upon the animal’s response or lack of response to therapy. At the end of the study, mean glucose levels in the various groups were between 76.0 and 80.6 mg/dl, which was still less than the baseline levels before the infection ($P \leq 0.01$).

**CSF protein concentrations.** CSF protein concentrations before infection were $21.5 \pm 2.7$, $20.8 \pm 4.8$, and $21.7 \pm 3.0$ mg/dl for PEG-, FCZ-, and TBF-treated animals, respectively (Fig. 3B). By 11 to 12 days postinfection, protein levels increased at least fivefold for all treatment groups. While CSF protein concentrations in PEG- and TBF-treated animals continued to increase, FCZ-treated animals showed a decline in CSF protein concentration 18 to 19 days postinfection, and the concentration continued to decrease on day 25 to 26 postinfection compared to those for both PEG- and TBF-treated animals ($P \leq 0.04$). Although the protein concentrations for FCZ-treated animals decreased with time, they did not return to the levels found before infection ($P = 0.02$). Not enough CSF from the remaining PEG-treated rabbits was available on days 32 to 33 postinfection to obtain protein concentrations.

**CFU in CSF.** More than 50% of the CSF specimens collected from rabbits treated with PEG or TBF were positive for *C. immitis* on culture on days 11 to 12 postinfection (Table 1). Although not statistically significant, the CFU of fewer FCZ-treated rabbits (13%) tested positive for *C. immitis* for this early sampling period. The number of positive CSF cultures decreased for PEG- and TBF-treated animals on days 18 to 19. None of the rabbits had culture-positive CSF after days 25 to 26 postinfection.

**Antibody titers.** Antibody was present in the serum of only one animal (the PEG-treated group) at days 8 to 11. On days 15 to 18, about half the animals in each group were seropositive (five of eight, two of four, and four of seven in the FCZ,

<table>
<thead>
<tr>
<th>TABLE 1. Fungal CFU in cultures of CSF</th>
<th>No. of cultures positive/total no. of cultures on the following days postinfection:</th>
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<tbody>
<tr>
<td>Treatment</td>
<td>11–12</td>
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<tr>
<td>-----------</td>
<td>-------</td>
</tr>
<tr>
<td>PEG</td>
<td>5/8</td>
</tr>
<tr>
<td>FCZ</td>
<td>1/8</td>
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<td>TBF</td>
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animals were sampled at the other times.

For assays with CSF, seven animals were sampled at day 26 and eight animals were sampled at each time point on day 25.5 h postdosing. For assays with serum, eight animals were sampled at each time point on day 25 postinfection.

PEG, and TBF groups, respectively), with all titers being <1:4 except in one PEG-treated animal, which had an immunoglobulin G (IgG) titer of 1:64 and an IgM titer of 1:4. Nine animals were IgG seropositive and four animals were IgM seropositive. On day 25, only two animals were then seronegative (one in the FCZ treatment group and one in the TBF treatment group); however, only three animals (one in each group) had titers of ≥1:16 for IgG, and only three animals were IgM seropositive. By days 27 to 32 only three animals, all in the FCZ group, were seronegative. In the latter group, of the five seropositive animals, four had titers of <1:16 and only one was IgM seropositive. In contrast, all surviving PEG- and TBF-treated animals were seropositive, with all but one animal having titers of ≥1:16, and IgM seropositivity persisted in more than half of them. Thus, serum antibody titers suggest a responsiveness to FCZ.

No animals had antibody in their CSF on days 8 to 11. On days 15 to 18, only two animals (in the FCZ group) were positive (IgG). However, by day 25, all PEG-treated animals and half the FCZ- and TBF-treated animals had antibody titers (IgG), which were low (<1:8); one TBF-treated animal, however, had a titer of 1:8 to 1:16. On days 27 to 32, all PEG- and TBF-treated animals were seropositive, with titers of 1:4 to 1:32 (IgG), whereas four of nine FCZ-animals remained seronegative. The only IgM titer (positive undiluted only) in any animal on any day was detected in the TBF-treated group on day 32. Thus, the CSF IgG antibody titers also reflected a more favorable overall response to FCZ.

**Drug levels.** FCZ levels in the serum and CSF at 24 to 25.5 h postdosing were well above the MIC for the C. immitis strain used in the study (Fig. 4). FCZ levels were generally 15 to 20% higher in serum than in CSF.

TBF levels in serum were measured during the study at 1 to 1.5, 2 to 2.5, 4, or 14 h postdosing (Fig. 5). Serum TBF levels were well above the MIC for C. immitis for over 4 h and were above or close to the MIC at 14 h. Peak serum drug levels were reached sooner as treatments continued. On day 25 postinfection, a sample obtained at 14 h postdosing showed high levels of TBF. No TBF was detected in the CSF at any of the time points.

**DISCUSSION**

The general decline in the numbers of CFU in CSF contrasts with the culture positivity of the brain and spinal cord, suggesting that the organism is embedded in the meningeal and parenchymal inflammatory reaction and is no longer liberated into the CSF. This evolution is the same as that in the human disease (2).

In this study, despite the in vitro susceptibility of the organism to TBF, TBF appeared to have only a modest effect on survival, histologic findings, and tissue CFU reduction; however, the effects were not significant. This leaves open the question of whether TBF may be effective when used in combination with other antifungals.

In a study with rats, TBF was shown to cross the blood-brain barrier at levels higher than expected from the free fraction available (7). Thus, it was conceivable that measurable amounts of TBF could cross the blood-brain barrier and decrease the fungal burden in a meningeal disease. In the study with rats, TBF was injected directly into the carotid artery, which may not stimulate the conditions encountered when the drug is given orally.

One could argue that the lack of measurable TBF in the CSF was responsible for the inferior performance of TBF compared to that of FCZ. However, in a previous study (13) we showed that penetration or accumulation of measurable itraconazole in the CSF was not needed for itraconazole to have efficacy equal to that of FCZ in this model. Presumably, the accumulation of itraconazole in the meninges is responsible for the efficacy. Like itraconazole, TBF is known to accumulate in tissues (6). The accumulation of TBF in adipose- and keratin-rich tissues at concentrations significantly higher than those in plasma are most likely responsible for a good response against superficial infections (1).

FCZ was effective at controlling coccidioidal meningitis and reducing C. immitis levels in the CSF and tissue. The antibody titers in serum and CSF were consistent with this. As was shown in a previous study with this model (13), FCZ penetrates the CSF at levels slightly below the levels obtained in serum. However, it did not eliminate C. immitis from the tissues. Although FCZ is efficacious in the treatment of human coccidioidal meningitis, it likewise appears not to be curative (2).
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