Efficacy of Intravenous Itraconazole against Experimental Pulmonary Aspergillosis

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The efficacy of intravenous itraconazole solubilized in hydroxypropyl-β-cyclodextrin was assessed in a rat model of Aspergillus fumigatus pneumonia. Immunosuppressed rats were infected by intratracheal inoculation of A. fumigatus conidia. Intravenous administration of various doses of itraconazole was started immediately after infection and continued once a day for 7 days. A 10-mg dose of intravenous itraconazole per kg was as effective on survival as 1 mg of amphotericin B per kg daily (a survival rate of 100% in 28 days), while treatment with 1 mg/kg did not increase the survival rate. The 50% lethal dose of intravenous itraconazole given to immunosuppressed and uninfected rats for 7 days was 24.5 mg/kg/day. A microbiological assay to estimate accumulation in tissue after five daily intravenous administrations of itraconazole at 10 mg/kg showed that itraconazole and its active metabolites were present in the lungs for at least 6 h, reaching the MIC as previously described (B. Dupont and E. Drouhet, Rev. Infect. Dis. 9(Suppl. 1):71–76, 1987; A. Espinel-Ingroff, S. Shadomy, and R. J. Gebhart, Antimicrob. Agents Chemother. 26:5–9, 1984). Intravenous itraconazole was considered to be worth evaluating in clinical trials of aspergillosis.

Itraconazole is a promising agent in treatment of aspergillosis because of its in vitro and in vivo activity against Aspergillus species (6, 7) and also because of its minimal toxicity (6, 21). Treatment with oral itraconazole against aspergillosis has been evaluated in both animal models and clinical studies (2, 4, 6, 9, 14, 25, 26). However, since levels of itraconazole in serum show a large degree of variation among patients (24), because absorption varies between individuals and may be influenced by foods (29), decisions regarding doses and evaluation of efficacy are problematic. In addition, oral treatment is sometimes difficult for the patients because of nausea, vomiting, or decreased mental ability. Therefore, intravenous administration of itraconazole should be evaluated as one approach to improving antifungal therapies. Hostetler et al. reported that hydroxypropyl-β-cyclodextrin made itraconazole, which had poor aqueous solubility because of its hydrophobic structures, soluble and enhanced oral itraconazole absorption, as determined by bioassay (11). We assessed the efficacy of intravenous administration of itraconazole against experimental invasive pulmonary aspergillosis by mortality in rats and studied the toxicity and levels of this agent in both serum and tissues by using immunosuppressed rats.

Efficacy of intravenously injected itraconazole. Experimental invasive aspergillosis was induced in rats as described by Schmitt et al. (22) with some modifications. Briefly, male Sprague-Dawley rats (Charles River, Kanagawa, Japan) weighing 130 to 150 g were immunosuppressed by subcutaneous injection of 90 mg of cortisone acetate (Banyu Pharmaceutical) per kg three times weekly and given a low-protein diet (8% protein; Oriental Yeast) to ensure a uniformly lethal infection. The injections and diet were continued for 1 week prior to infection plus the 1 week of antifungal therapy. Aspergillus fumigatus MF-13 isolated from sputum of a patient with a pulmonary aspergilloma was used for infection. The isolate was cultured on Sabouraud dextrose agar plates at 30°C for 4 days, and conidia were harvested with 0.02% Tween 80. After two washings in sterile saline, conidia were suspended in sterile saline and counted in a hemacytometer. Three days after the third cortisone acetate injection, the immunosuppressed rats were infected by intratracheal inoculation of 8 × 10³ spores contained in 0.1 ml of saline by tracheostomy under general anesthesia with enfurane (ethrane; Abbott). Drinking water with tetracycline (250 mg/800 ml) was given throughout the whole experiment to prevent bacterial infection.

Efficacies of the antifungal agents were evaluated as follows. Itraconazole solubilized in 40% hydroxypropyl-β-cyclodextrin solution at 10 mg/ml was a generous gift from Janssen Research Foundation, Beerse, Belgium. It was diluted to give the appropriate concentration in 0.3 ml of 40% hydroxypropyl-β-cyclodextrin solution. Amphotericin B (Squibb) diluted in 5% glucose solution was used for comparison. Treatments were given by daily intravenous administration in the tail vein starting immediately after infection, for 7 days. Six groups of five infected rats were treated as follows. The rats in the itraconazole treatment groups were given either 20, 10, 5, or 1 mg of itraconazole per kg per day. Those treated with amphotericin B were given 1 mg of amphotericin B per kg per day. Control rats were given 40% hydroxypropyl-β-cyclodextrin solution without itraconazole. Survival was observed in each group of five rats for 28 days.

Figure 1 shows the survival rates of infected rats in the treatment studies. The survival curves of the different groups were compared by use of the generalized Wilcoxon test. A P value of <0.05 was considered significant. All control rats given only 40% hydroxypropyl-β-cyclodextrin solution died by day 15. All deaths were due to invasive pulmonary aspergillosis, as determined by autopsy. There

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There was no microscopic or cultural evidence of infection in the livers, kidneys, spleens, or brains. Treatment with intravenous itraconazole at 20 and 10 and 5 mg/kg/day and amphotericin B for 7 days significantly prolonged survival (P < 0.01, < 0.05, and < 0.01, respectively, versus controls). The 20 and 10 mg/kg/day dosages, with a survival rate of 100%, were as effective as 1 mg of amphotericin B per kg per day. In contrast, treatment of rats with 1 mg of intravenous itraconazole per kg per day did not increase the survival rate. Histopathological studies of the lungs of all surviving rats on day 29 showed multiple and clearly encapsulated lesions with proliferation of Aspergillus hyphae in the center of the lesions (lung sections not shown).

**Itraconazole assay.** Rats immunosuppressed in the way described above and uninfected were given five daily doses of 10 mg of itraconazole per kg intravenously. Ten minutes, 6 h, and 24 h after the last administration, three rats from each group were sacrificed and then blood, lungs, livers, kidneys, and spleens were obtained. Lungs, livers, kidneys, and spleens were homogenized with 1 ml of 0.9% NaCl, and supernatants of homogenates were used for bioassay.

Microbiological studies were performed according to the method of Warnock et al. (27). Candida albicans (7N strain) cells from cultures grown on Sabouraud dextrose agar plates were suspended in sterile water and adjusted to a density of

10⁶ cells per ml. Ten-milliliter amounts of concentrated Bacto Yeast Nitrogen Base solution (67 g/liter; Difco) (pH 7.0) supplemented with glucose (100 g/liter) and trisodium citrate (59 g/liter) were added to 90-ml amounts of molten cooled purified agar (20 g/liter; Oxoid) dispensed into 23-cm square plates. Twenty milliliters of a C. albicans suspension was poured onto the surface of each plate, and then the excess was poured off and the plate was dried at 37°C. On each plate, wells 8 mm in diameter were cut out and filled with standard or test samples. A standard curve was made by using known concentrations of drug ranging from 0.01 to 100 μg/ml. Serum was undiluted. Each specimen and each standard was tested in triplicate. The plates were incubated overnight at 37°C, and the diameters of the zones of inhibition were then measured.

As shown in Table 1, the concentrations in serum were 1.596 ± 0.240 μg/ml 10 min after injection. The levels in lungs 10 min and 6 h after injection were 0.453 ± 0.096 μg/g and 0.451 ± 0.101 μg/g, respectively, higher than in livers and kidneys but lower than in spleens. At 24 h after injection, itraconazole was not detected in lungs by bioassay.

**Toxicity of itraconazole.** Immunosuppressed rats were given 10, 20, 30, or 40 mg of itraconazole per kg in 0.6 ml of 40% hydroxypropyl-β-cyclodextrin solution by one-shot daily intravenous injections in the tail veins for 7 days. The animals were not infected. Survival was observed in each group of five rats for 28 days. On the days the drug was started and discontinued, body weights were compared with those of rats administered 1 mg of amphotericin B per kg per day.

![FIG. 1. Cumulative mortality of rats with invasive pulmonary aspergillosis in treatment and control groups. Treatments were given by daily intravenous administration, starting immediately after infection, for 7 days. Symbols: ○, itraconazole at 10 or 20 mg/kg/day; □, itraconazole at 5 mg/kg/day; ○, itraconazole at 1 mg/kg/day; Δ, amphotericin B at 1 mg/kg/day; ×, control (0.3 ml of 40% hydroxypropyl-β-cyclodextrin solution).](image1)

![FIG. 2. Results of toxicity study of survival of treatment with intravenous itraconazole once daily for 7 days at various dosages. Symbols: △, itraconazole at 10 or 20 mg/kg/day; □, itraconazole at 30 mg/kg/day; ○, itraconazole at 40 mg/kg/day.](image2)

### TABLE 1. Concentrations of itraconazole and its active metabolites in sera and tissues of rats after seven daily administrations of 10 mg of itraconazole per kg intravenously

<table>
<thead>
<tr>
<th>Time after injection</th>
<th>Drug concn* in:</th>
<th>Serum</th>
<th>Lungs</th>
<th>Livers</th>
<th>Spleens</th>
<th>Kidneys</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Drug concn* in:</td>
<td>10 min</td>
<td>6 h</td>
<td>24 h</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.596 ± 0.240</td>
<td>0.975 ± 0.278</td>
<td>0.258 ± 0.172</td>
<td>N/D</td>
<td>N/D</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.453 ± 0.096</td>
<td>0.451 ± 0.101</td>
<td>0.201 ± 0.110</td>
<td>0.854 ± 0.183</td>
<td>0.264 ± 0.084</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.182 ± 0.057</td>
<td>0.225 ± 0.074</td>
<td>0.258 ± 0.092</td>
<td>0.695 ± 0.182</td>
<td>0.225 ± 0.057</td>
</tr>
</tbody>
</table>

* Values are means ± standard deviations. Concentrations in serum are measured in micrograms per milliliter, and concentrations in tissues are measured in micrograms per gram. N/D, not detectable.
The survival kinetics of rats in the dose-response studies of intravenous itraconazole are shown in Fig. 2. All rats in the 10- and 20-mg/kg/day administration groups survived to day 29. However, in the 30- and 40-mg/kg/day groups, all animals died by day 8. The 50% lethal dose for 7-day intravenous administration of itraconazole was 24.5 mg/kg/day as determined by the method of Kärber (12). On the day treatment was discontinued, rats given 20 mg of itraconazole per kg per day showed weight gain (mean gain of 3.3%) while rats given 1 mg of amphotericin B per kg per day showed weight loss (mean loss of 5.8%), though the difference was not significant.

Invasive pulmonary aspergillosis is an infection caused by opportunistic fungi. Despite improvements in diagnosis (5, 8, 10, 15, 20, 28, 30) and treatment (1, 4, 13, 16, 18, 22), invasive aspergillosis in immunocompromised patients is still a life-threatening disease. New antifungal therapies with increased efficacy and decreased toxicity are needed to improve its management. If itraconazole intravenously administered can achieve the necessary concentration, this drug may prove to be efficacious in treatment of patients.

Our therapeutic study indicated that intravenous itraconazole was effective in a model of invasive aspergillosis and prolonged survival. Graybill and Ahrens reported that oral itraconazole given to mice infected intranasally with conidia of *A. fumigatus* neither prolonged survival nor lowered counts of *A. fumigatus* in lung tissue compared with those in control mice, in contrast to oral itraconazole in intravenously infected mice, which prolonged survival over that in controls and produced lower counts in the kidney (9).

Although we cannot compare our data to theirs because we did not use the same model, the findings of our study, employing intravenous therapy of rats infected intratracheally with conidia, are encouraging. However, histopathological studies of the lungs of surviving rats showed that seven daily intravenous injections of 20 mg of itraconazole per kg had not completely eradicated the *Aspergillus* hyphae after 4 weeks.

In our toxicity study, 20 mg/kg for 7 days was the maximum dosage to be safely administered to rats, as determined by survival rate, but nonfatal side effects were not investigated. Our study of toxicity was performed with immunosuppressed rats, and the drug was administered in a single shot. In the foundation experiments, we confirmed that the immunosuppression we used is not lethal for 30 days without infection or treatment. It is possible, however, that the immunosuppression and the rapid rate of injection of the drug contributed to the apparent toxicity. Sharkey et al. reported that toxicity is common with clinical doses of more than 400 mg of oral itraconazole per day (23).

The MIC of itraconazole against *Aspergillus* spp. was reported by Espinel-Ingroff et al. to be about 0.13 μg/ml (7), and it was reported by Dupont and Drouhet to range from <0.09 to 0.36 μg/ml (6). Concentrations of itraconazole in lungs reached the MIC and were higher than concentrations in the liver and kidneys at 10 min and 6 h after injection but lower than those in the spleen. Microbiological assay measures both the native drug and the hydroxy metabolite, preventing a direct comparison to the MIC. These properties suggest that itraconazole may be more beneficial in treatment of pulmonary infections such as aspergillosis than amphotericin B, which shows lower concentrations in lungs than in the liver and spleen in animal studies (3, 19).

In conclusion, intravenous itraconazole effectively prolonged survival in an immunosuppressed rat model of invasive pulmonary aspergillosis. Further extensive studies could assess the potential of intravenous itraconazole as an alternative to amphotericin B for therapy of aspergillosis.

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