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Activity of ICI 195,739, a New Oral Triazole, Compared with That of Ketoconazole in the Therapy of Experimental Murine Blastomycosis

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ICI 195,739, a novel orally active triazole, proved 50 times as potent as ketoconazole, produced a clinical cure, and completely eradicated residual infection in a murine model of pulmonary blastomycosis. No other previously tested azole has shown similar activity. Fungicidal activity against *Blastomyces dermatitidis* was seen in vitro at concentrations 1/40 of those achieved in serum with protective doses.

We previously described a murine model of pulmonary blastomycosis that has proven a useful means of evaluating new antifungal agents for possible human use (3). Ketoconazole (KTZ) (4) and itraconazole (E. G. Arathoon, E. Brummer, and D. A. Stevens, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, F18, p. 392) have shown activity in this model and have both had documented efficacy in the therapy of human blastomycosis (1, 6). ICI 195,739 (ICI) is a recently developed oral bis-triazole (Fig. 1). We have examined its efficacy in comparison with that of KTZ in this model in order to evaluate its potential benefit in the treatment of human fungal infection.

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Methods. Seven-week-old Sendai virus-free male BALB/cByJIMR mice weighing 20.0 ± 1.3 (standard deviation) g were obtained from the breeding colony at the Institute for Medical Research, San Jose, Calif. Acidified water and sterilized food and bedding were provided. ICI was provided by Imperial Chemical Industries Pharmaceuticals, Macclesfield, Cheshire, United Kingdom, and KTZ was provided by Janssen Pharmaceutica, Beerse, Belgium.

A virulent strain of *Blastomyces dermatitidis* (ATCC 26199) grown in a defined liquid medium on a shaker at 35°C for 3 days was used to inoculate a blood agar plate (3). A saline suspension of washed organisms grown on blood agar for 3 days at 35°C was prepared to provide an estimated 1,000-CFU inoculum per mouse. This inoculum was verified to be 778 CFU per mouse by quantitative culture on blood agar. Mice were lightly anesthetized with ether and given an intranasal challenge of 30 μl of the inoculum.

In vitro susceptibility studies were performed for both ICI and KTZ against this organism and for ICI against one additional reference strain (ATCC 26197), a variant of ATCC 26199 maintained in our laboratory, and two patient isolates of *B. dermatitidis*. ICI was solubilized in dimethyl sulfoxide and diluted in culture medium. The concentration of dimethyl sulfoxide in the range of concentration of drug assayed was noninhibitory. KTZ was prepared, and MICs and minimal fungicidal concentrations (MFCs) were determined for both drugs by a previously described tube dilution method, followed by subculture to agar (2).

Treatment groups consisted of 10 mice each given oral KTZ suspended in 1% agar (4) by gavage at 10 or 100 mg/kg of body weight per day divided into two daily doses or ICI suspended in 0.5% Tween 80 in normal saline, given orally by gavage at 2, 10, 50, or 100 mg/kg per day once daily. The KTZ dosage regimen was derived from earlier efficacy and pharmacokinetic studies (4). Control groups consisted of 20 uninfected mice given ICI (100 or 50 mg/kg per day) and 10 infected mice gavaged once daily with 0.5% Tween 80 in normal saline (placebo control). Therapy began 3 days after infection and continued daily for 21 days. Cages were examined twice daily to monitor mortality.

Ten uninfected controls treated with ICI at 50 mg/kg per day were sacrificed after the final dose (day 21) to provide chronic pharmacokinetic data. Two mice were sacrificed at 2, 4, 8, 24, and 48 h after dosing. Twenty mice were used for acute pharmacokinetic studies after a single ICI dose of 50 mg/kg. Two mice were sacrificed at 0.5, 1, 2, 4, 8, 24, 48, 96, and 168 h after dosing. Sera from the two mice sacrificed at each time point were pooled, and levels of ICI were measured by an agar bioassay, with *Candida pseudotropicalis* as a test organism, as previously described (6).

All surviving animals were sacrificed by cervical dislocation after deep ether anesthesia 60 days after inoculation. Both lungs were dissected and homogenized in saline. Quantitative cultures of this homogenate were made on sheep erythrocyte agar (3). The sensitivity of this assay is approximately 1 CFU of *B. dermatitidis* per 40 mg of lung tissue, or 5 CFU per mouse. Survival data were analyzed by the Wilcoxon rank sum test. Significance was assumed to be $P < 0.05$.

In vitro results. For the organism used in the in vivo studies, the MIC and MFC of ICI were 0.25 and 0.5 μg/ml, respectively, and the MIC and MFC of KTZ were 0.78 μg/ml. Among the remaining four isolates, the MICs of ICI were ≤0.125 μg/ml for three and 0.25 μg/ml for one; the MFCs of ICI were ≤0.125 μg/ml for one, 0.25 μg/ml for one, and 0.5 μg/ml for two.

In vivo results. Cumulative mortality in the treatment

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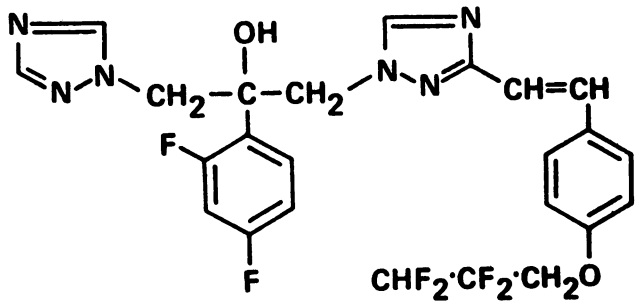

FIG. 1. Chemical structure of ICI 195,739.

groups is shown in Fig. 2. Survivals were identical in the placebo group and the treatment group receiving KTZ at 10 mg/kg per day; all of these animals died between 15 and 25 days after infection. This result is the same as previously reported for this model (4); in the earlier study, it was demonstrated that KTZ at 10 mg/kg per day and no treatment or gavage with 1% agar daily gave identical results. Mice treated with ICI at 2 mg/kg per day had a longer median survival than did those treated with KTZ at 100 mg/kg per day (37 versus 31 days), but this difference was not statistically significant ($P > 0.05$). Each dose was superior to the placebo control and KTZ at 10 mg/kg per day ($P < 0.01$). All animals in these groups, however, died of infection between 29 and 57 days after inoculation. Mice dying during therapy or during the posttherapy period were selected randomly for autopsy. Results of gross pathologic examination of these mice were indistinguishable from those seen previously in this model (3) and documented by histopathology and culture to be associated with overwhelming fungal infection.

None of the animals treated with ICI at 10, 50, or 100 mg/kg per day died during the 60-day observation period, a result significantly superior to those obtained for the treatment groups receiving KTZ at 100 mg/kg per day and ICI at 2 mg/kg per day. These surviving animals were sacrificed 60 days after infection so that lungs could be cultured. None of the infected mice or controls treated with ICI at 50 or 100 mg/kg per day showed overt signs of drug toxicity.

Quantitative cultures of lung homogenates were obtained from each of the 10 surviving mice in the groups receiving ICI at 10, 50, and 100 mg/kg per day. All except one from an animal in the group receiving ICI at 10 mg/kg per day were sterile. Lung homogenates from this animal contained 1.34×10^3 CFU. This animal was also the only sacrificed animal that showed any gross evidence of pulmonary infection: a solitary 1-mm lung abscess of the right lower lobe.

Pharmacokinetics. The pharmacokinetics of ICI after a single 50-mg dose and in the steady state (50 mg/day) are shown in Fig. 3. After the single dose, a peak of 17.6 μg/ml occurred at 12 h postdosage; the half-life was prolonged (>12 h). In the steady state, a trough of 19.5 μg/ml occurred at 4 h and a peak of 21.0 μg/ml occurred at 8 h. The half-life was markedly prolonged (≥48 h).

Treatment of chronic, deep mycoses with presently available azoles has been marked, on occasion, by disease progression and relapse despite prolonged therapy (1, 5). Such failures suggest a lack of in vivo fungicidal activity. This deficiency has been demonstrated previously in this model of pulmonary blastomycosis. Although KTZ, itraconazole, or fluconazole has provided significant protection from mortality, none has provided protection equal to that seen with amphotericin B. Only amphotericin B and its derivatives have shown consistent in vivo fungicidal activity (4).

We have documented that ICI, like KTZ, is fungicidal in vitro against *B. dermatitidis* and is more than 50 times as potent as KTZ in vivo. Unlike KTZ, ICI provides complete protection from mortality in this murine model of blastomycosis. Perhaps most important, ICI exhibits apparent fungi-

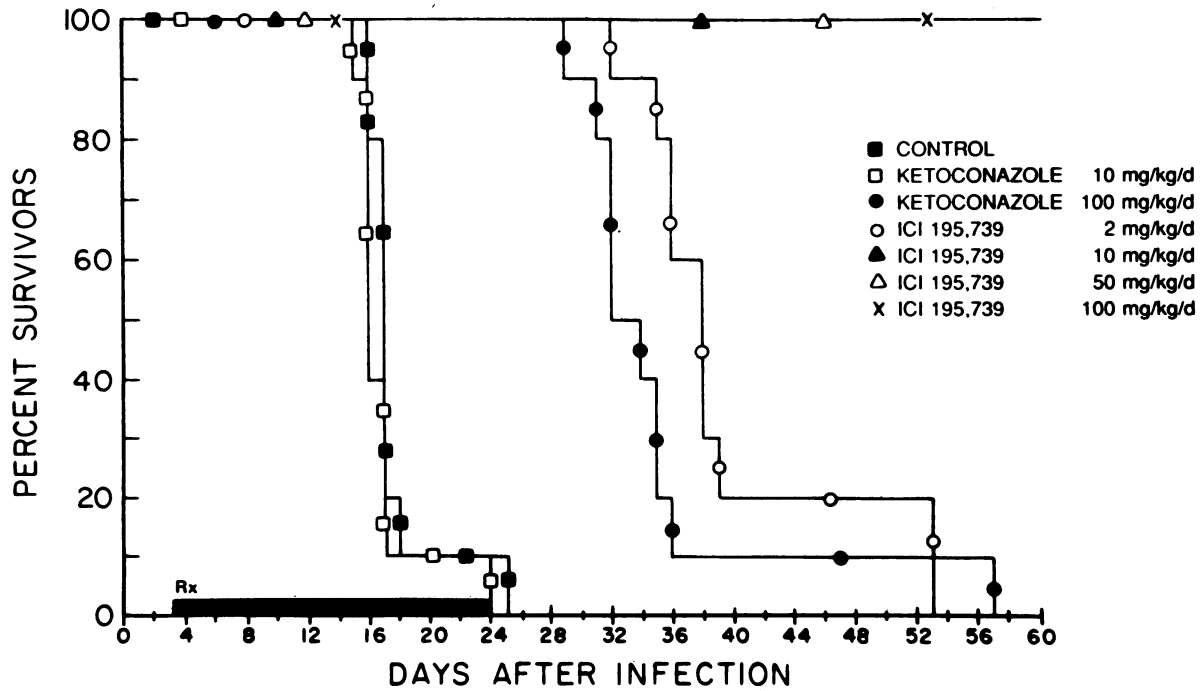

FIG. 2. Cumulative mortality of mice infected with *B. dermatitidis*.

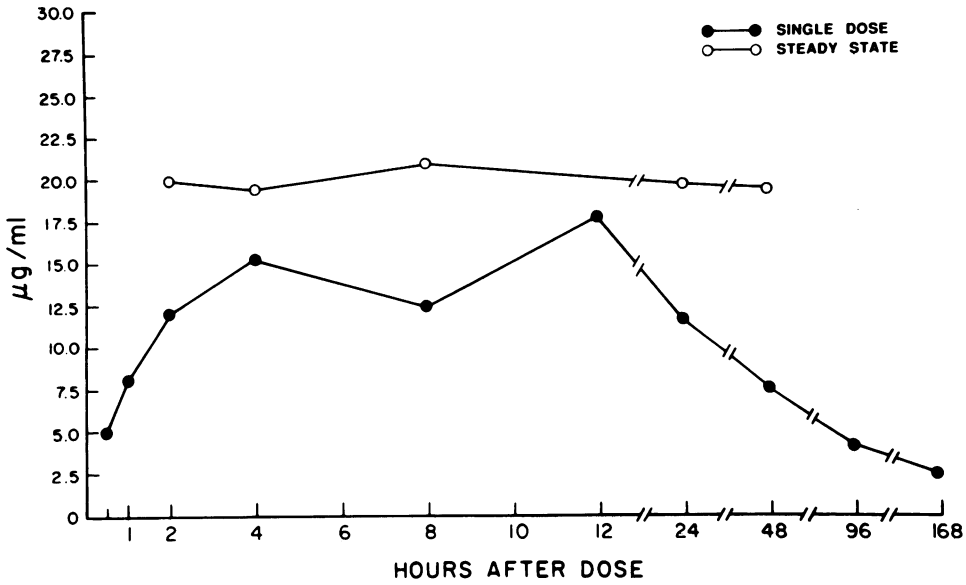

FIG. 3. Pharmacokinetics of ICI 195,739 in adult mice.

cidal activity against *B. dermatitidis* in vivo. This efficacy was demonstrated in a highly lethal infection (equal to or greater than the 100% lethal dose). ICI has a very long half-life in serum in this model, and residual levels of the drug in tissues may have affected cultures of lung homogenates. Lung cultures were obtained, however, 36 days after the last dose of ICI. Moreover, the dilution factor from lung to agar for any hypothesized residual drug was approximately 500-fold. Further evidence that ICI cured the infection is that all ICI-treated animals, except one treated at a low dose, showed no evidence of disease after extended follow-up.

Ryley et al. have demonstrated the efficacy of ICI in murine models of systemic candidiasis, cryptococcosis, and aspergillosis (4a). In addition, ICI shows trypanosomacidal activity in vivo against *Trypanosoma cruzi*. The broad-spectrum in vitro antifungal activity of ICI and its ability to sterilize fungal infection in vivo justifies further evaluation of this compound or analogs in animal models and consideration for initial clinical trials in refractory human mycoses.

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