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Itraconazole for Experimental Pulmonary Aspergillosis: Comparison with Amphotericin B, Interaction with Cyclosporin A, and Correlation between Therapeutic Response and Itraconazole Concentrations in Plasma

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Itraconazole and amphotericin B were compared by using a newly developed model of invasive pulmonary aspergillosis in rabbits immunosuppressed with methylprednisolone and cyclosporin A (CsA). Both itraconazole at 40 mg/kg (given orally) and amphotericin B at 1 mg/kg (given intravenously) had in vivo antifungal activity in comparison with controls. At these dosages, amphotericin B was more effective than itraconazole in reducing the tissue burden ($\log_{10}$ CFU per gram) of *Aspergillus fumigatus* ($P < 0.05$) and the number of pulmonary lesions ($P < 0.01$). However, there was considerable variation in the near-peak concentrations of itraconazole in plasma (median, 4.15 μg/ml; range, <0.5 to 16.8 μg/ml) and a strong inverse correlation between concentrations of itraconazole in plasma and the tissue burden of *A. fumigatus*. An inhibitory sigmoid maximum-effect model predicted a significant pharmacodynamic relationship ($r = 0.87$, $P < 0.001$) between itraconazole concentrations in plasma and antifungal activity as a function of the tissue burden of *A. fumigatus*. This model demonstrated that levels in plasma of greater than 6 μg/ml were associated with a significantly greater antifungal effect. Levels in plasma of less than 6 μg/ml were associated with a rapid decline in the antifungal effect. Itraconazole, in comparison with amphotericin B, caused a twofold elevation of CsA levels ($P < 0.01$) but was less nephrotoxic ($P < 0.01$). This study of experimental pulmonary aspergillosis demonstrated that amphotericin B at 1 mg/kg/day was more active but more nephrotoxic than itraconazole at 40 mg/kg/day, that itraconazole increased concentrations of CsA in plasma, and that the antifungal activity of itraconazole strongly correlated with concentrations in plasma in an inhibitory sigmoid maximum-effect model. These findings further indicate the importance of monitoring concentrations of itraconazole in plasma as a guide to increasing dosage, improving bioavailability, and optimizing antifungal efficacy in the treatment of invasive pulmonary aspergillosis.

Invasive pulmonary aspergillosis is a life-threatening and increasingly recognized infection in immunosuppressed patients, particularly in those with granulocytopenia or those undergoing organ or bone marrow transplantation (1, 22, 24, 31). The cornerstone of treatment of invasive pulmonary aspergillosis in immunosuppressed hosts is administration of high dosages of desoxycholate amphotericin B (2, 4). Unfortunately, successful treatment of this devastating infection is severely complicated in many patients by the development of dose-limiting nephrotoxicity. Treatment with amphotericin B is also hampered in transplant patients by potentially synergistic toxicity of this antifungal agent and cyclosporin A (CsA) (13).

There is a great need for safe and effective antifungal compounds against invasive aspergillosis. Itraconazole is a recently introduced antifungal agent with good activity against *Aspergillus* spp. (8, 29). Itraconazole has not been compared with amphotericin B against invasive pulmonary aspergillosis in randomized clinical trials, although results of initial nonrandomized studies appear promising (6, 7, 30). However, among the concerns with antifungal azole derivatives, such as itraconazole, is their potential adverse interaction with CsA that may increase CsA levels, resulting also in nephrotoxicity (15, 16, 26), and their limited bioavailability in some patients that may lower concentrations in serum (6, 25, 27).

Thus, to understand the comparative safety and efficacy of itraconazole compared with amphotericin B in transplant recipients more thoroughly, we evaluated these compounds in a newly developed model of invasive pulmonary aspergillosis in rabbits immunosuppressed with methylprednisolone and CsA. This model reflects the immunosuppressive modalities and pharmacological interactions encountered in transplant recipients.

MATERIALS AND METHODS

Animals. Female New Zealand White rabbits (TSI-Washington Vienna, Va.) weighing between 2 and 3 kg at the time of inoculation were used in all of the experiments. They were housed and maintained in accordance with National Institutes of Health guidelines for animal care and in fulfillment of American Association for Accreditation of Laboratory Animal Care criteria. Vascular access was established in each rabbit by

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surgical placement of a Silastic tunnelled central venous catheter (32).

Immunosuppression. Rabbits were immunosuppressed by a combination of CsA plus methylprednisolone given intravenously on a daily basis. CsA (Sandimmune ampoules for injection; Sandoz Pharmaceuticals Corporation, East Hanover, N.J.) was dissolved at 2.5 mg/ml in normal saline, stored protected from light at 4°C, and used within 24 h. The dose was 10 mg/kg/day at a rate of 0.5 mg/kg each 15 s. Methylprednisolone (Solu-Medrol; Upjohn Co., Kalamazoo, Mich.) was dissolved in normal saline at a concentration of 6.25 or 3.57 mg/ml, stored at room temperature, and used within 48 h. The methylprednisolone doses used were 5 mg/kg/day during the first 3 days and then 2 mg/kg/day thereafter.

Fungus and inoculum. Rabbits received an intratracheal inoculum of 2.8×10^8 conidia of *Aspergillus fumigatus* on day 2 of the experiment after the second dose of the immunosuppressive combination. A strain of *A. fumigatus* (isolate 4215) obtained from a fatal case of pulmonary aspergillosis was used in all of the experiments. Testing of the susceptibility of this isolate to both itraconazole and amphotericin B was performed by broth macrodilution with an inoculum of 1×10^4 to 5×10^4 CFU/ml in RPMI 1640 medium buffered with 0.165 M MOPS [3-(*N*-morpholino)propanesulfonic acid] containing L-glutamine and lacking sodium bicarbonate (BioWhittaker, Inc., Walkersville, Md.). Tubes were read after 48 h of incubation at 35°C. The MIC was defined as the lowest concentration of an antifungal compound which rendered no growth (0) to slight growth (1+) on a 0-to-4+ scale. The minimum lethal concentration was determined by dispensing and streaking 100 μl of broth from tubes exhibiting no growth onto Sabouraud dextrose agar (Media Department, National Institutes of Health, Bethesda, Md.) and incubating it at 35°C. The minimum lethal concentration was defined as the lowest concentration of an antifungal compound with growth of ≤3 colonies. With this method, MICs and minimum lethal concentrations of amphotericin B were 0.5 and 8 μg/ml, respectively, and those of itraconazole were 0.25 and >16 μg/ml. CsA concentrations of up to 160 μg/ml had no effect on the growth of this strain of *A. fumigatus* (14, 20).

The inoculum of *A. fumigatus* was prepared from a frozen isolate that was subcultured onto potato dextrose agar slants, which were incubated for 24 h at 37°C and then kept at room temperature for 5 days. Conidia were harvested under a laminar air flow hood with a solution of 0.025% Tween 20 (Fisher Scientific, Fair Lawn, N.J.) in normal saline, transferred to a 50-ml conical tube, and counted in a hemacytometer. The concentration was adjusted to give each rabbit a predetermined inoculum of 2.8×10^8 *A. fumigatus* conidia in a volume of 200 to 350 μl. The concentrations of inocula were confirmed by serial dilution and culture on Sabouraud dextrose agar check plates.

Inoculation of rabbits was performed on day 2 of the experiments under general anesthesia with 0.5 to 1.0 ml (intravenous [i.v.]) of a 2:1 (vol/vol) mixture of 100-mg/ml ketamine (Fort Dodge Labs, Fort Dodge, Iowa) and 20-mg/ml xylazine (Mobay Corp., Shawnee, Kans.). Once satisfactory anesthesia was reached, a Flagg 0 straight-blade laryngoscope (Welch-Allyn, Skaneateles Falls, N.Y.) was inserted until the vocal cords were clearly visualized and the *A. fumigatus* inoculum was administered intratracheally with a tuberculin syringe attached to a 5.25-in. Teflon catheter (Becton Dickinson, Sandy, Utah).

Antifungal therapy. Amphotericin B (Fungizone; Bristol Myers-Squibb, Princeton, N.J.) was reconstituted with distilled water, maintained at 4°C, and diluted 1:5 with 5% dextrose in water to a final concentration of 1 mg/ml immediately prior to use. Itraconazole (Sporanox; 100-mg capsules; Janssen Pharmaceutica, Titusville, N.J.) was pulverized with a 75-W blender (Chemical Rubber Co., Cleveland, Ohio) and suspended in 50% light corn syrup to a final concentration of 25 mg/ml. Beginning 1 day after inoculation, rabbits were treated daily with either amphotericin B at 1 mg/kg (i.v.; $n = 12$) or itraconazole at 40 mg/kg (orally; $n = 14$) in parallel with untreated, infected control animals ($n = 9$).

Outcome variables. On day 11 postinoculation, rabbits were euthanized by pentobarbital anesthesia. This immunosuppressed rabbit model of invasive pulmonary aspergillosis was designed to provide survival of animals through 11 days of infection. All of the experiments were evaluated on the basis of the following outcome variables.

(i) Lung weight. At autopsy, the tracheobronchial tree and lungs were carefully dissected and weighed (Mettler Instrument Co., Hightstown, N.J.).

(ii) Pulmonary lesion score. The lungs were inspected by two observers who were blinded to the treatment group of the specimens. These observers recorded the type of lesions, if any, in each separate lobe. Pulmonary lesions in this model were classified as (i) hemorrhagic infarcts (dark red consolidated lesions that corresponded histologically to coagulative necrosis and intraalveolar hemorrhage), (ii) neutrophilic lesions (firm, aggregated or confluent grayish-white nodular lesions that corresponded histologically to foci of neutrophilic infiltrates with necrosis), and (iii) monocytic lesions (firm, confluent, flattened, grayish-red lesions with a translucent quality, that corresponded histologically to zones of chronic mononuclear inflammation and fibrosis without necrosis). Lobes found to have more than one type of lesion were scored for each type identified. This scoring system is intentionally stringent, such that a lobe containing even one lesion is scored as positive.

(iii) Fungal cultures. Bronchoalveolar lavage (BAL) was performed three times on each lung preparation by instillation and subsequent withdrawal of 10 ml of sterile normal saline into a clamped trachea, and 0.1-ml samples of this fluid were cultured on Sabouraud dextrose agar and Trypticase soy agar, respectively. Thereafter, the most abnormal region in each lobe was excised for cultures and histopathologic examination. Each fragment was weighed individually, minced with sterile scissors, and homogenized with sterile saline for 1 min per tissue sample (Stomacher 80; Tekmar, Cincinnati, Ohio). Lung homogenates in 10^{-2} and 10^{-4} dilutions were prepared in sterile saline, and 100-μl aliquots were plated onto Sabouraud dextrose agar and incubated at 37°C for the first 24 h and then at room temperature for another 24 h. After this, the CFU of *A. fumigatus* were counted and recorded for each lobe and the CFU per gram of tissue were calculated. A finding of one colony of *A. fumigatus* was considered positive. In addition, the percentage of lobes that were culture negative was calculated for each rabbit.

(iv) Blood chemistry analysis. A sample of blood was collected from each rabbit on day 1 of each experiment and thereafter every other day until death or sacrifice. Samples were stored in Sarsted tubes (Sarsted, Inc., Newton, N.C.) at −70°C until all samples were processed simultaneously. Chemical determinations included blood urea nitrogen, serum creatinine, and alanine aminotransferase (Anilytics, Gaithersburg, Md.).

(v) Levels of itraconazole in plasma. Plasma samples for determination of itraconazole levels were drawn 2 h after administration of the last oral dose on the day before sacrifice. This permitted a "near-peak" level of itraconazole to be obtained for correlation with concentrations of *A. fumigatus* in

tissue (log_{10} CFU per gram). Autopsies and cultures of lung tissue were performed 24 h after the last dose of itraconazole was administered. The near-peak levels of this last dose of itraconazole in serum were correlated with the concentrations of *A. fumigatus* in pulmonary tissue.

Levels of itraconazole in plasma were determined by an agar bioassay using *Candida kefyr* (ATCC 46764) as the test organism. The coefficients of variance of the inter- and intraday variations of the assay were 2.3 to 3.9% over the range of concentrations from 0.5 to 20 μg/ml. The linear regression curve followed a function of $y = [0.37\ (\pm 0.09)x] + 18\ (\pm 0.87)$, where $r = 0.92$. The bioassay measures total itraconazole antifungal activity, including that due to hydroxyitraconazole and itraconazole (the parent compound).

Itraconazole concentrations in serum and the corresponding *Aspergillus* concentrations in tissue expressed as log_{10} CFU per gram per lobe were fitted to an inhibitory sigmoid maximum-effect model (18) by using nonlinear, least-squares regression with PCNONLIN (SCI Software, Lexington, Ky.). This relationship was defined by the equation $E = E_{max} \cdot \{1 - [C^{\gamma}/(C^{\gamma} + EC_{50}^{\gamma})]\}$, where E is the effect (tissue burden) observed at concentration C, E_{max} is the maximum effect when no drug is present, EC_{50} is the concentration in serum which produces a 50% decrease in effect, and γ is a constant which characterizes the shape of the concentration-effect curve.

(vi) Levels of CsA blood. Levels of CsA in whole blood were determined by high-performance liquid chromatography for the parent compound. The detection limit of this method was 20 ng/ml, with a between-runs coefficient of variation of 5.3% ($n = 23$) for a 200-ng/ml control (American Medical Laboratories, Inc., Chantilly, Va.).

Statistical analysis. Comparisons between proportions were done by the χ^2 or Fisher exact test, as appropriate. Comparisons of numerical variables for three independent groups were performed by one-way analysis of variance and the Student Newman Keuls test or the Kruskall Wallis procedure followed by the Dunn test, as appropriate. The coefficient of correlation between itraconazole levels in plasma and the tissue burden of *A. fumigatus* was calculated by the Spearman rho rank correlation.

RESULTS

As shown in Fig. 1, rabbits treated with either amphotericin B or itraconazole had fewer neutrophilic lesions ($P < 0.001$) but more monocytic lesions ($P \leq 0.01$) than did untreated controls. The reduction of neutrophilic lesions was more marked in rabbits treated with amphotericin B (3%) than in rabbits treated with itraconazole (18%) ($P < 0.01$). The lungs of the untreated controls were approximately 1.5 times heavier than the lungs from rabbits receiving antifungal therapy ($P < 0.01$), reflecting differences in inflammatory infiltration of the lungs between untreated and treated animals (Table 1).

At least 1 CFU of *A. fumigatus* was isolated from 100% of both controls and itraconazole-treated rabbits versus 17% of amphotericin B-treated animals ($P < 0.001$). However, both amphotericin B and itraconazole were effective against invasive pulmonary aspergillosis according to the results of qualitative and quantitative cultures of lung tissue (Table 1). Amphotericin B was, nevertheless, more effective than itraconazole in reducing the tissue burden of *A. fumigatus* ($P < 0.05$) and the proportion of lobes with infection ($P < 0.001$). As shown in Table 2, BAL fluid cultures yielded *A. fumigatus* in 78% of untreated control animals versus 16% of those treated with amphotericin B ($P = 0.001$). In comparison with 78% of untreated rabbits with *A. fumigatus* in cultures of BAL fluid,

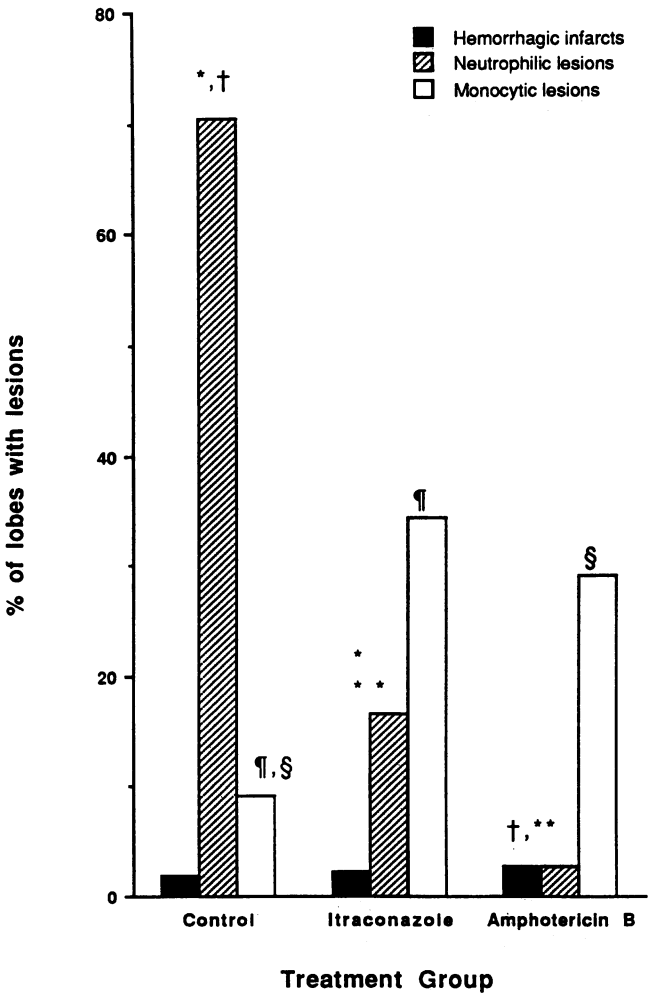

FIG. 1. Pulmonary lesion scores in experimental invasive pulmonary aspergillosis. Rabbits treated with either amphotericin B or itraconazole had fewer neutrophilic lesions and more monocytic lesions than did untreated controls, reflecting a reparative response. The reduction of neutrophilic lesions was greater in rabbits treated with amphotericin B than in rabbits treated with itraconazole. Symbols: *, †, and ¶, $P < 0.001$ for these pairs; § and **, $P = 0.01$.

36% of animals treated with itraconazole had culture-positive BAL fluid ($P = 0.123$).

The median peak concentration of itraconazole in plasma after nine doses was 4.15 μg/ml, with values ranging from less than 0.5 to 16.8 μg/ml. As shown in Fig. 2, a significant correlation ($r = 0.87$, $P < 0.001$) was observed between itraconazole concentrations in plasma and tissue burden in an inhibitory sigmoid maximum-effect model. Increases in itraconazole levels in serum resulted in decreases in the tissue burden by an inhibitory sigmoidal relationship with an EC_{50} of 3.72 μg/ml. Levels in plasma of less than approximately 5 μg/ml demonstrated a precipitous decline in antifungal activity over a narrow range of itraconazole concentrations. By comparison, levels in plasma of greater than 6 μg/ml demonstrated a sustained diminution of *Aspergillus* levels in tissue over a wide range of concentrations. The model predicted that absence of a drug would result in a tissue burden of 2.9 log_{10} CFU/g per lobe (E_{max}). The model was strongly predictive of the actual mean *Aspergillus* tissue burden of 2.99 ± 0.26 log_{10} CFU/g per lobe obtained in untreated control animals (Table 1).

TABLE 1. Effect of amphotericin B versus that of itraconazole on microbiological clearance, nephrotoxicity, and CsA levels in primary pulmonary aspergillosis in rabbits immunosuppressed with CsA plus methylprednisolone

Treatment (no. of rabbits)	% of lobes culture positive	Mean $\log_{10}$ CFU/g/lobe ± SEM	Mean lung wt (g) ± SEM	Mean CsA trough level in whole blood (ng/ml) ± SEM	Mean blood urea nitrogen (mg/dl) ± SEM	Mean serum creatinine (mg/dl) ± SEM
None (9)	74[a,b]	2.99 ± 0.26[a]	16.9 ± 2.7[a]	51.4 ± 7.5[c]	14.4 ± 0.6[c]	0.72 ± 0.03[a]
Itraconazole (14)	56[a,b]	1.52 ± 0.17[a,d]	11.3 ± 1.1[a]	128 ± 16.8[c]	15.7 ± 0.8[e]	0.6 ± 0.03[a,c]
Amphotericin B (12)	3[a,b]	0.06 ± 0.04[a,d]	11.0 ± 0.8[a]	54.7 ± 11.3[c]	33.3 ± 6.6[c,e]	0.89 ± 0.05[a,c]

[a] $P < 0.05$ for comparison of either treatment group with untreated controls.
[b] $P < 0.001$.
[c] $P < 0.01$.
[d] $P < 0.05$.
[e] $P < 0.01$.

Trough levels of CsA in whole blood, determined by high-performance liquid chromatography on study day 10, were approximately twice as high in rabbits treated with itraconazole as in those treated with amphotericin B or in untreated, infected controls ($P < 0.01$) (Table 1). The blood urea nitrogen and creatinine determinations obtained 24 h before euthanasia were significantly greater in rabbits treated with amphotericin B than in rabbits treated with itraconazole ($P < 0.01$) (Table 1). In comparison with untreated controls, the serum creatinine in itraconazole-treated animals also was significantly reduced.

DISCUSSION

This study found that itraconazole and amphotericin B had in vivo antifungal activity in a model of invasive pulmonary aspergillosis in rabbits immunosuppressed by a combination of methylprednisolone and CsA. Amphotericin B was more effective than itraconazole both in reducing the number of neutrophilic lesions and in clearing *A. fumigatus* from lung tissue. There was a strong inverse correlation between concentrations of itraconazole in plasma and the tissue burden of *A. fumigatus*. Rabbits treated with itraconazole had a twofold elevation of CsA in comparison with those treated with amphotericin B and with untreated controls. There was no increased nephrotoxicity due to this interaction between itraconazole and CsA. By comparison, amphotericin B did not increase CsA levels but was more nephrotoxic than itraconazole.

This model of invasive pulmonary aspergillosis is the first system that experimentally reproduces the immune deficits observed in patients receiving the commonly used immunosuppressive drug combination of CsA and methylprednisolone for transplantation. This offers a unique opportunity to study the patterns of infection and response to antifungal drugs in invasive pulmonary aspergillosis as they occur in transplant recipients and to study the interaction between CsA and antifungal drugs. This nonlethal model of invasive pulmonary aspergillosis also permits comparisons of the therapeutic efficacy of antifungal compounds and immunomodulatory agents at the same time points. The presence of a central silastic venous catheter permits nontraumatic venous access for i.v. drug administration and repeated blood sampling.

In this study, itraconazole proved to be an effective drug against invasive pulmonary aspergillosis. However, amphotericin B was more effective than itraconazole at sterilizing BAL and tissue cultures and reducing the tissue burden of *A. fumigatus*. Previously published experimental studies have shown that itraconazole can prolong survival in corticosteroid-treated mice challenged i.v. with aspergilli in comparison with that of controls (10, 29). In a model of invasive pulmonary aspergillosis in corticosteroid-immunosuppressed rats, there was significant prolongation of survival of animals treated orally with itraconazole at 40 or 80 mg/kg/day over controls but not over animals treated intraperitoneally with amphotericin B at 4 mg/kg/day (23). In a study published by Graybill and Ahrens (9), both itraconazole at 70 mg/kg given orally twice daily and amphotericin B at 3 mg/kg given intraperitoneally 3 days per week prolonged survival and reduced the kidney

TABLE 2. Cultures of BAL fluid in experimental invasive pulmonary aspergillosis

Treatment (no. of rabbits)	No. (%) of rabbits with culture-positive BAL fluid	Mean concn (CFU/ml) of *A. fumigatus* in BAL fluid ± SEM
None (9)	7 (78)[a,b]	34.4 ± 22.3[c,d]
Itraconazole (14)	5 (36)[a,e]	17.0 ± 4.5[d,f]
Amphotericin B (12)	0 (0)[b,e]	0[c,f]

[a] $P = 0.123$.
[b] $P = 0.001$.
[c] $P < 0.001$.
[d] $P > 0.05$.
[e] $P = 0.071$.
[f] $P > 0.002$.

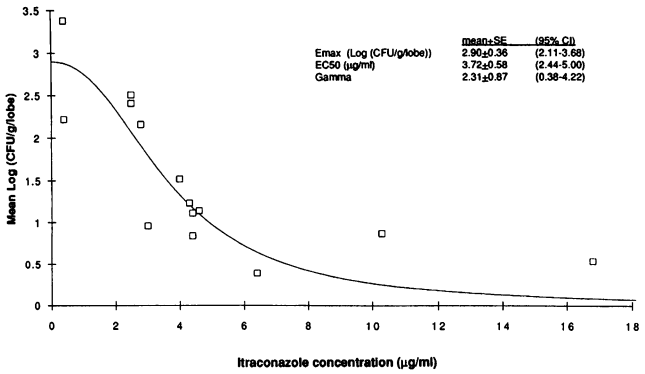

FIG. 2. Inhibitory sigmoid maximum-effect model predicts a significant pharmacodynamic relationship ($r = 0.87$, $P < 0.001$) between itraconazole concentrations in plasma and antifungal activity as a function of the tissue burden ($\log_{10}$ CFU/gram per lobe) of *A. fumigatus*. SE, standard error; CI, confidence interval; EC50, concentration in serum that produced a 50% decrease in the effect; Emax, maximum effect with no drug present.

tissue burden of aspergilli in comparison with controls. However, mice challenged intranasally had neither longer survival times nor lower lung tissue counts than did controls.

There are no reported randomized trials comparing itraconazole and amphotericin B for the treatment of invasive pulmonary aspergillosis. However, results of initial studies of itraconazole against invasive pulmonary aspergillosis appear promising, particularly for organ transplant recipients (3, 6, 7, 30). A randomized trial comparing amphotericin B and itraconazole is currently being conducted in the United States.

There was a strong correlation in this study between peak itraconazole concentrations in plasma and the *Aspergillus* burden in tissue. There also was a striking interindividual variation of itraconazole levels in plasma in this otherwise homogeneous group of animals with closely controlled immunosuppression and dietary intake. This variation appears to have significant therapeutic implications. Previous studies with humans also have revealed marked variability of drug levels in serum among patients treated with itraconazole, and low levels have been implicated as potential causes of therapeutic failure (5, 6). Tricot et al. (27), in a study in which itraconazole (200 mg/day, given orally) was used for prophylaxis of fungal infections in granulocytopenic patients, found that 21 (47%) of 45 patients receiving the drug had persistently inadequate levels of itraconazole in serum. They also found a significantly lower proportion of proven or suspected fungal infections in patients with adequate levels of itraconazole. These researchers mentioned poor compliance with oral medication due to nausea and vomiting as the most likely explanation for the low levels in serum. The absorption of orally administered itraconazole is reduced in AIDS patients, by a factor of approximately 50%, compared with normal volunteers (25). Another factor that may preclude adequate absorption of itraconazole in immunocompromised hosts is taking the drug in the fasting state or by nasogastric tube (6, 11). In addition to impaired bioavailability, individual variations in levels of itraconazole in plasma may be due to the compound's Michaelis-Menten saturable plasma pharmacokinetics. Concurrent therapy with rifampin, phenytoin, and carbamazepine also can lower the levels of itraconazole in serum by induction of hepatic enzymes that metabolize itraconazole (28).

The sigmoidal shape of the pharmacodynamic relationship between itraconazole levels and the tissue burden (Fig. 2) may represent an important concept in understanding triazole therapy in immunosuppressed hosts. The model suggests that a critical range of itraconazole concentrations in serum was required for optimal effectiveness. Concentrations at the bottom of the curve (greater than approximately 6 μg/ml) achieved considerable antifungal activity, whereas in the sigmoidal section, small decreases in the level in serum below this value resulted in considerable loss of in vivo activity. Antifungal activity in animals with concentrations in plasma of >6 μg/ml approximated that of amphotericin B. These data provide support for therapeutic monitoring of itraconazole in immunosuppressed patients. In addition, these findings suggest that increasing the dosage or improving the absorption of itraconazole to more effective concentrations may improve efficacy in the treatment of invasive aspergillosis in the setting of low levels in serum.

Monitoring of itraconazole concentrations in plasma may permit recognition of patients with impaired bioavailability and low circulating levels of itraconazole. Increasing the dosage of itraconazole may permit higher levels to be achieved in patients with low levels and impaired bioavailability (21). Potential lines of pharmaceutical research for increasing the bioavailability of itraconazole include incorporation into cyclodextrins as drug carriers for oral or i.v. administration (11, 12) or formulation of the compound into liposomes for i.v. administration (17). While our study correlated near-peak concentrations in plasma with antifungal activity, there may be substantial covariance with other dynamic covariables, such as the area under the concentration-time curve and time spent above the MIC. Further studies with more intensive sampling and different dosage schedules are needed to further understand the pharmacodynamics of itraconazole against invasive pulmonary aspergillosis.

A significant pharmacokinetic interaction between itraconazole and CsA was found in this study, with levels of CsA twofold higher in animals treated with itraconazole than in controls or amphotericin B-treated animals. Case reports or small series of patients have also demonstrated this interaction in renal, heart, and lung transplant recipients, with an associated deterioration in renal function induced by CsA (15, 16, 26). However, one study failed to observe itraconazole and CsA interaction in patients with bone marrow transplantation during an average time of 4.4 weeks in which both drugs were administered together (19). The latter findings may be explained in part by the frequent occurrence of low levels of itraconazole in the serum of granulocytopenic patients, especially those with mucosal disruption, poor oral intake, and frequent vomiting associated with cytotoxic chemotherapy (27).

Increased levels of CsA during concurrent administration of itraconazole may be due to competitive inhibition of hepatic mixed-function oxidases, resulting in impaired metabolism of CsA and increased levels of the parent compound in blood. Elevation of CsA in this study was not associated with excess nephrotoxicity. While there was a greater-than-twofold elevation of trough CsA levels in itraconazole-treated animals in comparison with those of other groups, these levels were still within a range considered to be only minimally nephrotoxic for short-term (2 weeks) exposure. If the trough levels greatly exceeded 150 μg/ml during the 2-week study period, increased nephrotoxicity would likely have occurred.

Amphotericin B proved to be more nephrotoxic than itraconazole in this study. The differences in urea nitrogen and creatinine reported for amphotericin B revealed a significant pharmacological effect, albeit not a clinically significant effect. In other experiments (data not shown), when rabbits were treated for 28 days, greater levels of azotemia ensued to approach clinically significant renal insufficiency (>50% increase in serum creatinine). This lack of nephrotoxic potential is a clear advantage of itraconazole over amphotericin B, especially in the setting of organ transplantation, because of the potentially synergistic nephrotoxic interaction between CsA and amphotericin B (13). Itraconazole-induced elevated CsA levels have been reported to cause nephrotoxicity. CsA dosages during itraconazole therapy may be practically managed by serial monitoring of CsA levels and appropriate adjustment of the CsA dosage before sustained nephrotoxic CsA levels develop. Despite the higher levels of CsA in the itraconazole-treated group, there was a decline in serum creatinine in animals receiving itraconazole. Untreated controls in these experiments demonstrated a slight increase in serum creatinine from the baseline. This increment in serum creatinine may be related to production of cytokines, such as tumor necrosis factor alpha, in response to untreated invasive pulmonary aspergillosis. Itraconazole exerted a nonnephrotoxic reduction of invasive aspergillosis and a trend toward reversal of elevated serum creatinine, possibly reducing the stimulus for cytokine production. Yet another other possible mechanisms to account for the decline of serum creatinine in

itraconazole-treated animals may be the inhibition of formation of possibly nephrotoxic CsA metabolites.

Perhaps the development of parenteral formulations of itraconazole or of more readily bioavailable formulations of itraconazole for oral administration (e.g., cyclodextrin solution) will provide circulating levels with greater antifungal activity. The strong pharmacodynamic correlation between concentrations of the drug in plasma and therapeutic efficacy underscores the necessity of monitoring levels of the drug.

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