Pharmacodynamics of Itraconazole Against *Aspergillus fumigatus* in an In Vitro Model of the Human Alveolus: Perspectives on the Treatment of Triazole Resistant Infection and Utility of Airway Administration

Zaid Al-Nakeeb, Ajay Sudan, Adam R. Jeans, Lea Gregson, Joanne Goodwin, Peter A. Warn, Timothy W. Felton, Susan J. Howard and William W. Hope

School of Translational Medicine, The University of Manchester, Manchester Academic Health Science Centre, National Institute for Health Research Translational Research Facility in Respiratory Medicine, University Hospital of South Manchester National Health Service, Foundation Trust, Manchester, United Kingdom

Running title = Pharmacodynamics of Itraconazole

Abstract word count = 250
Corresponding author
Professor William Hope
1.800 Stopford Building
The University of Manchester
Oxford Rd., Manchester, M13 9PT
United Kingdom
+44 (0)161 275 3918
william.hope@manchester.ac.uk

KEY WORDS
Itraconazole, pharmacokinetics, pharmacodynamics, triazole, Aspergillus, resistance

Acknowledgments
William Hope is supported by a National Institute of Health (NIHR) Clinician Scientist Fellowship.
Timothy Felton is supported by an Medical Research Council (MRC) Fellowship in Clinical Pharmacology
Abstract

Introduction: Itraconazole is used for the prevention and treatment of infections caused by Aspergillus fumigatus. An understanding of the pharmacodynamics of itraconazole against wild-type and triazole resistant strains provides a basis for innovative therapeutic strategies for treatment of infections.

Methods: An in vitro model of the human alveolus was used to define the pharmacodynamics of itraconazole. Galactomannan was used as a biomarker. The effect of systemic and airway administration of itraconazole was assessed as well as a combination of itraconazole administered to the airway and systemically administered 5FC.

Results: Systemically administered itraconazole against the wild-type induced a concentration-dependent decline in galactomannan in the alveolar and endothelial compartments. No exposure-response relationships were apparent for the L98H, M220T or G138C mutants. The administration of itraconazole to the airway resulted in comparable exposure-response relationships to those observed with systemic therapy. This was achieved without detectable concentrations of drug within the endothelial compartment. The airway administration of itraconazole resulted in a definite, but sub-maximal effect in the endothelial compartment against the L98H mutant. The administration of 5FC resulted in a concentration-dependent decline in galactomannan in both the alveolar and endothelial compartments. The combination of airway administration of itraconazole and systemically administered 5FC was additive.

Conclusions: Systemic administration of itraconazole is ineffective against Cyp51 mutants. The airway administration of itraconazole is as effective for treatment of wild-type strains and appears also to have some activity against the L98H mutants. Combination with other agents, such as 5FC may enable the attainment of near maximal antifungal activity.
Introduction

Aspergillus fumigatus is a leading cause of pulmonary fungal infections in immunocompromised patients. Invasive pulmonary aspergillosis (IPA) remains a significant public health problem. There are relatively few therapeutic options. Little is known about the pharmacodynamics of antifungal agents against Aspergillus spp. An improved understanding of pharmacokinetic and pharmacodynamic relationships represents a first critical step towards further optimization of antifungal therapy for patients with IPA.

Itraconazole is a broad spectrum triazole agent with potent activity against Aspergillus spp. (5). Itraconazole was the first orally bioavailable antifungal agent with activity against Aspergillus spp. (6). Itraconazole has an established role for the prevention of Aspergillus infections (15, 16). While there are data to support the use of itraconazole for acute invasive aspergillosis, newer triazoles are generally used for this indication. Itraconazole can be used for the treatment of chronic pulmonary aspergillosis and allergic bronchopulmonary aspergillosis (ABPA) (5). The clinical utility of itraconazole capsules is somewhat hampered by its relatively poor oral bioavailability; this prompted the development of novel formulations. Aerosolized therapy potentially circumvents some of the problems of poor oral bioavailability and enables the attainment of effective concentrations at the site of infection. The utility of itraconazole is also threatened by the emergence of triazole resistance (11). Relatively little is known about the pharmacodynamics of itraconazole against these resistant isolates. Innovative therapeutic strategies are urgently required to provide viable therapeutic options.

Here, we use a well-validated in vitro model of the human alveolus (9) to describe the pharmacodynamics of itraconazole against both wild-type and resistant strains of Aspergillus fumigatus. This model has enabled us to explore some potential advantages of innovative therapeutic strategies, such as administration of drug to the airway and combining airway administration of itraconazole with flucytosine (5FC), an agent that is usually not considered to have an anti-Aspergillus effect. This study provides some insights into potential therapeutic opportunities for patients with resistant Aspergillus who ordinarily have severely limited treatment options.
MATERIALS AND METHODS

Isolates of *Aspergillus fumigatus* and In Vitro Susceptibility Testing.

The initial pharmacodynamic experiments were performed using a green fluorescent protein (GFP) transformant of *Aspergillus fumigatus* as previously described (9, 12, 13). While we did not perform any imaging in this study, we chose this strain because it has been extensively characterized in this in vitro model. Subsequently, three additional clinical isolates (F/11628, F/14532 and F/16216) with elevated minimum inhibitory concentrations (MICs) to itraconazole were obtained from the Regional Mycology Reference Laboratory, University Hospital South Manchester. The putative molecular basis for the reduced in vitro susceptibility to itraconazole in these strains is related to amino acid substitutions in the triazole target protein Cyp51, as previously described (11); see Table 1. Itraconazole MICs were determined using The European Committee on Antimicrobial Susceptibility Testing (EUCAST) and Clinical and Laboratory Research Institute (CLSI) methodologies (1, 3). MICs were estimated with three independently conducted experiments.

In vitro Model of the Human Alveolus.

A previously described cell culture model of the human alveolus was used to investigate the pharmacodynamics of itraconazole against susceptible and resistant strains of *Aspergillus fumigatus* (9). Briefly, this model consists of a cellular bilayer with a monolayer of alveolar epithelial cells (A549; LGC Standards, Teddington, United Kingdom) and human pulmonary artery endothelial cells (HPAECs; Lonza Biologics, Slough, UK). Both monolayers are seeded on a Transwell polyester membrane with 3 µm perforations (Corning, Lowell, USA). The cellular bilayer delineates an alveolar compartment (air-space) and an endothelial compartment (pulmonary vasculature). Itraconazole was administered into the endothelial compartment (to mimic systemic drug administration) or within the alveolar compartment to mimic aerosolized therapy. Antifungal activity was estimated using galactomannan, as previously described (9). Evidence from both laboratory animal models and clinical settings suggest that concentrations of galactomannan are inextricably related to the pathogenesis of invasive pulmonary aspergillosis and the ultimate prognosis (10). Sampling from the in vitro model was destructive (i.e. each insert contributed a single terminal sample meaning that sampling did not have an impact upon the estimates of the pharmacokinetics and pharmacodynamics of itraconazole).
The inoculum for each strain was prepared as previously described (9, 12, 13). One-hundred µL of a 1x10^4 cfu/mL conidial suspension (i.e. 1x10^3 conidia) was added to the alveolar compartment and incubated for 6-hours. The final inoculum was based on a previous study where the time-course of infection following several different inocula was determined (9). Subsequently, the medium containing the inoculum was removed and replaced with fresh medium. The inserts were then incubated for a further 18-hours. All experiments finished 24-hours post inoculation. The 6-hour delay in drug administration was chosen in order to replicate early treatment of IPA, and was based upon previous studies (9). All experiments were conducted at least twice.

**Antifungal Agents**

Pure itraconazole powder (Sigma) was dissolved in dimethyl sulfoxide (DMSO; Sigma) to produce a stock solution of 1600 mg/L. A range of dilutions were created by serially diluting the stock concentration in DMSO, before a single step dilution (1:100) into EBM-2 (Lonza Biologics) supplemented with 2% fetal bovine serum (FBS; Lonza Biologics). The FBS enabled protein binding to occur as is the case in laboratory animals and humans. Depending on the experiment, itraconazole was added to either the alveolar or endothelial compartment. Pure flucytosine powder (Sigma) was dissolved in dimethyl sulfoxide (DMSO) to produce a stock solution of 6400 mg/L. A range of dilutions were created by serially diluting the stock solution into EBM-2 supplemented with 2% FBS. Flucytosine was only administered to the endothelial compartment.

**Measurement of Galactomannan**

The fungal burden was estimated by measuring galactomannan, which is a large molecular weight antigen secreted by hyphal phase of *Aspergillus* (9, 10). GM was measured using a double-sandwich enzyme-linked immunosorbent assay (ELISA) (Platelia *Aspergillus* kit; Bio-Rad Laboratories, Hemel Hempstead, UK) according to the manufacturer’s instructions with a single modification to the volume of the sample, as previously described (12).

**High Performance Liquid Chromatography (HPLC)**

Itraconazole concentrations were measured using HPLC with a Shimadzu Prominence (Shimadzu, Milton Keynes, UK) and a Kinetex C18 2.6 µL column 75x4.6mm (Phenomenex, UK).
The injection volume was 40 µL. A standard curve encompassing 0.125–8 mg/L was constructed in the respective matrix, from stock solutions of itraconazole 1000 mg/L in DMSO (Fisher Scientific, Loughborough, UK). The internal standard was diazepam 1 mg/L (Sigma) in acetonitrile. The starting mobile phase was 70% 0.1% trifluoroacetic acid (TFA; Fisher) in water (v/v) and 30% 0.1% TFA in acetonitrile (v/v) (Fisher) with a gradient over 6.25 minutes progressing to 30% over 4 minutes and flow rate of 1 mL/min. Itraconazole and the internal standard were detected using UV 254 nm; they eluted after 4.6 and 2.9 minutes, respectively. The CV% was <6% over the concentration range 0.125 – 8 mg/L. The limit of detection was 0.125 mg/L. The intra and inter-day variation was <7%.

Pharmacokinetics of Itraconazole following systemic and aerosolized therapy

Itraconazole was added to either the alveolar or endothelial compartment to mimic airway administration or systemic drug administration, respectively. In the case of airway administration, an initial concentration of itraconazole in the alveolar compartment in the range 0.5-8 mg/L was used. The pharmacokinetics in both compartments were estimated. Itraconazole was added 6 hrs post inoculation. Samples were taken at 6, 8, 10, 12 and 24 hrs post inoculation. For the alveolar compartment, this was achieved by adding 300 µL of PBS to mimic a bronchoalveolar lavage.

Pharmacodynamics of Itraconazole Following Systemic and Aerosolized Therapy

The exposure-response relationships for itraconazole against the wild-type (GFP transformant) following the systemic and airway administration of itraconazole were initially determined. Galactomannan concentrations were measured in both the alveolar and endothelial compartments at the end of the experimental period (i.e. 24-hrs. post inoculation, 18 hours post initiation of antifungal therapy. The initial itraconazole concentration in the alveolar and endothelial compartments ranged from 0-8 mg/L. Subsequently, the exposure-response relationships for the three additional strains with reduced susceptibility to itraconazole were determined. The same sampling strategy and itraconazole dosages that were used for the wild-type were studied. The impact of systemic versus airway administration was determined.
Pharmacodynamics of 5FC Alone and in Combination With Airway Administration of Itraconazole.

Flucytosine was administered to the endothelial compartment to achieve clinically relevant initial concentrations in the range 0-120 mg/L. The experimental design was the same as described above. Only a single strain was studied (L98H). Galactomannan concentrations in both the endothelial and alveolar compartments were estimated at the end of the experiment, 24 hours post inoculation and 18 hours post initiation of drug therapy.

The combination of systemically administered 5FC and airway administration of itraconazole against L98 was studied. The combination matrix was designed with prior information related to the exposure-response relationships for each modality alone (obtained from the experiments described above). Itraconazole was administered to the alveolar compartment to achieve initial concentrations of 0-8 mg/L. Simultaneously, 5FC was administered to the endothelial compartment to achieve concentrations 0-120 mg/L. Combinations of these regimens were then studied.

Data Analysis and Mathematical Modeling

The pharmacodynamic data were modeled using an inhibitory sigmoid Emax model that was implemented in the pharmacokinetic program ADAPT 5 (4). The data were weighted by the inverse of the observed variance estimated from three inserts. This model takes the form:

\[ E = E_{\text{con}} - \frac{E_{\text{max}} \times (\text{exposure})^H}{IC_{50} + \text{exposure}^H} \]

Where \( E_{\text{con}} \) is the galactomannan concentration in the absence of therapy, \( E_{\text{max}} \) is the asymptotic reduction in galactomannan concentration induced by itraconazole, \( E_{50} \) is the concentration of itraconazole that induced half-maximal effect, and \( H \) is the slope (or Hill) function. The mean galactomannan values were weighted by the observed variance.

The combination of the airway administration of itraconazole and systemic 5FC was modeled using the Greco model, which is in turn based upon the concept of Loewe additivity. This model takes the form:

\[ 1 = \frac{D_{\text{ITRA}} \times \left( \frac{E}{E_{\text{con}} - E} \right)^{1/3max}}{IC_{50,\text{ITRA}} \times \left( \frac{E}{E_{\text{con}} - E} \right)^{1/3max}} + \frac{D_{\text{5FC}} \times \left( \frac{E}{E_{\text{con}} - E} \right)^{1/2max} + 1/3max}}{IC_{50,\text{5FC}} \times \left( \frac{E}{E_{\text{con}} - E} \right)^{1/2max} + 1/3max}} \]

\[ + \frac{\alpha \times D_{\text{ITRA}} \times D_{\text{5FC}}}{IC_{50,\text{ITRA}} \times IC_{50,\text{5FC}} \times \left( \frac{E}{E_{\text{con}} - E} \right)^{1/2max} + 1/3max}} \]
Where Econ is the galactomannan concentration in controls (i.e. absence of therapy), D_{ITRA} and D_{5FC} are the concentrations of itraconazole and flucytosine respectively, that produce effect, E; m_{ITRA} and m_{5FC} are the respective slope parameters for the two drugs; IC_{50,ITRA} is the initial concentration for itraconazole that produces 50% of the maximum effect; IC_{50,5FC} is the initial concentration of 5FC that produces 50% of the maximum effect; and α is the interaction parameter.

The first two terms on the right side of the equation describe the effect of itraconazole and 5FC alone (Loewe additivity); the third is the interaction term and contains the interaction parameter, α. If α is positive, but the 95% confidence interval crosses zero, the combined effect is additive. If α is positive and the lower bound of its 95% confidence interval excludes zero, synergy is present. If α is negative and the upper bound of its 95% CI excludes zero, antagonism is present.
RESULTS

Isolates and MICs
The MICs using CLSI and EUCAST methodology for all strains used in this study are summarized in Table 1. Of note, the 5FC MICs were >256 mg/L, which did not correlate with the exposure-response relationships that were subsequently observed (see below).

Pharmacokinetics of Itraconazole Following Administration to the Alveolar and Endothelial Compartments
The pharmacokinetics of itraconazole when administered to the endothelial compartment demonstrated a biphasic decline in concentrations in that compartment. In this in vitro model (i.e. cell culture medium was not added or removed), the change in concentrations with time reflects distribution of itraconazole into the cellular bilayer and contiguous compartments. Over the course of the experimental period, itraconazole concentrations progressively increased in the alveolar compartment (Figure 1). The pharmacokinetics for airway administration were different. While there was biphasic decline in the alveolar compartment, there were no quantifiable itraconazole concentrations in the endothelial compartment, presumably because of the discrepancy in volume between these compartments (Figure 1).

Pharmacodynamics of Itraconazole Against Susceptible and Resistant Strains of Aspergillus fumigatus
The systemic administration of itraconazole versus the susceptible GFP transformant induced a concentration-dependent decline in galactomannan concentrations in both the alveolar and endothelial compartments (Figure 2). The E50 (95% confidence interval) for the alveolar and endothelial compartments was 1.25 (0.79-1.70) and 0.44 (0.42-0.46) mg/L, respectively. There was a sub-maximal decline in galactomannan in the alveolar compartment. The airway administration of itraconazole resulted in comparable exposure response relationships (Figure 3). In this case the E50 (95% confidence interval) for the alveolar and endothelial compartments was 2.00 (1.13-2.89) and 0.65 (0.53-0.77) mg/L, respectively.

Interestingly, there was a comparable antifungal effect for systemic versus airway administration.
administration of itraconazole, but the latter was achieved without detectable drug concentrations in the endothelial compartment (as shown in Figure 1).

There was no demonstrable exposure response relationship for itraconazole against any of the strains with elevated MICs when itraconazole was administered to the endothelial compartment (Figure 4). The upper concentration used in these experiments was limited by the relatively low solubility of itraconazole (∼10 mg/L). In contrast, however, an exposure-response relationship was apparent when itraconazole was administered to the L98 mutant in the alveolar compartment. While there was no apparent effect on galactomannan concentrations in the alveolar compartment, there was a definite, albeit incomplete reduction of galactomannan concentrations in the endothelial compartment (Figure 5 Panel B).

**Pharmacodynamics of Systemically Administered 5FC**

The exposure response relationships in the alveolar and endothelial compartments with flucytosine were defined using an initial concentration of 0-120 mg/L. 5FC induced relatively languid exposure response relationships in both compartments (Figure 6). The E₅₀ (95% confidence interval) for the alveolar and endothelial compartments was 109.00 (94.30-123.80) and 76.59 (72.91-80.27) mg/L, respectively. This exposure response relationship was apparent despite the flucytosine MIC>256 mg/L.

**Pharmacodynamics of Systemically Administered 5FC in Combination with Itraconazole Administered to the Airway**

Both inhaled itraconazole and systemically administered 5FC induced a decline in galactomannan that was consistent with previous experiments where these modalities were studied alone against the L98H mutant (see Figures 4 and 5). The combination produced an additional decline in galactomannan to those observed when with either modality alone. The estimate for alpha, the interaction term in the Greco model was exceedingly small, and its 95% confidence interval crossed zero (Table 2). By definition, therefore, the interaction between systemically administered 5FC and itraconazole administered to the alveolar compartment was additive. The parameter values obtained from the fit of the Greco model to the data are shown in Table 2. The fit of the model to the data was excellent with a coefficient of determination of 0.90. The fitted surface is shown in Figure 7. The residuals (observed-predicted values) were relatively small and were normally distributed (data not shown).
IPA is a leading cause of infectious morbidity and mortality in immunocompromised patients (17). Itraconazole remains an important agent for the prevention of IPA and the treatment of some Aspergillus related lung diseases (5). Triazole resistance in Aspergillus is a potential looming public health crisis. The incidence of itraconazole resistance is approximately 6% in some European centers (11, 18). An important mechanism of reduced susceptibility of Aspergillus to the triazoles is related to amino-acid substitutions within the target protein Cyp51. An understanding of the pharmacodynamics of itraconazole against Aspergillus and potential therapeutic approaches are urgently required.

Using a well-validated in vitro model of the human alveolus, we have demonstrated that progressively higher itraconazole exposure against a wild-type strain of Aspergillus fumigatus induces a progressive decline in galactomannan concentrations. Near maximal decline in the biomarker was achieved using initial concentrations of ∼0.5 mg/L, which is comparable to plasma trough targets often recommended for patients receiving itraconazole (7). At these concentrations there was incomplete suppression in the alveolar compartment, raising the possibility that the organism is effectively treated in the vascular space, but persists in the alveolar compartment. Importantly, however, this conclusion should be qualified by our inability to model the antifungal effect of hydroxyl-itraconazole (the active metabolite of itraconazole), and the absence of pulmonary alveolar macrophages in this in vitro model. We could not demonstrate any exposure response relationship for well-characterized isolates with elevated itraconazole MICs and amino-acid substitutions in the triazole target protein when itraconazole was administered to the endothelial compartment. We studied concentrations as high as 8 mg/L, which would ordinarily be toxic in humans and which approaches the solubility limit for this compound. This experimental evidence suggests that dosage escalation and the achievement of higher itraconazole plasma concentrations is unlikely to be successful for the treatment of infections caused by these pathogens.

The cell culture model has the advantage of delineating two separate compartments that mimic the alveolar compartment and vasculature in humans. These compartments behave in a pharmacologically distinct manner. Administration of itraconazole to the alveolar compartment elicits comparable exposure response relationships and estimates for the EC50 to those following systemic administration. Importantly, however, the pharmacokinetic experiments showed that there were undetectable drug concentrations in the endothelial
compartment, and in this regard our findings are similar to findings in mice (8). These experiments therefore suggest that the administration of itraconazole to the airway may induce an important antifungal effect while minimizing systemic drug exposure, which is itself related to clinical toxicity and one of the significant limitations of this triazole (14). We were surprised to find evidence for at least some antifungal activity of itraconazole against the L98 mutant when administered to the alveolar compartment. While the decline in galactomannan in the endothelial compartment was sub-maximal, there was clearly some antifungal activity in this compartment. The reasons for this are not clear, especially since the concentrations used in the pharmacodynamic experiments were lower than the MIC. One potential explanation is the intercalation of itraconazole, which is lipid soluble compound, in the cell membranes of the alveolar epithelial cells. The cell membrane is ultimately an important component of the phagolysosome that is important for the killing of conidia. The phagocytic function and relative efficiency of A549 cells in comparison with macrophages has been previously described (21, 22). The achievement of high concentrations in this sub-compartment may far exceed the MIC and exert at least some antifungal activity. A similar concept has been recently described for posaconazole (2).

The observation of at least some, albeit sub-maximal antifungal effect following administration of itraconazole to the alveolar compartment therapy prompted a combination study. One problem for the treatment of patients triazole resistant infections is the absence of an orally bioavailable compound. While a polyene or an echinocandin could both be used for induction therapy, neither provide a realistic option for longer term consolidation therapy. We chose to study 5FC, which is usually reserved for the treatment of some recalcitrant Candida infections and for induction therapy for cryptococcal meningitis (5). One potential advantage of this compound is the availability of an oral formulation. Despite the elevated MIC of > 256 mg/L an exposure response relationship was observed. The reason for the discordance may be related to the use of an endpoint for MIC determination that does not require complete inhibition of growth. Furthermore, in vitro activity of flucytosine against Aspergillus is improved at a pH of 5 versus 7, which better predicts the activity that is observed with this compound in mice with disseminated aspergillosis (19, 20). Using interaction modeling, the combination of aerosolized itraconazole and systemically administered 5FC is additive against an L98H mutant. While not synergistic, the additive interaction may still be clinically beneficial to help secure a maximal therapeutic effect, and deserves further study with additional strains in laboratory
animal models and in clinical contexts. The combination of the agents helps “complete” the inherent deficiency of itraconazole (related to the Cyp51 substitution) and the upper toxicity bound of 5FC. Additional combination regimens with various aerosolized compounds and systemic administration of agents that are not ordinarily classical anti-Aspergillus agents may be feasible and deserves further study.

There are a number of limitations of this study. Firstly, this is a model of early invasive aspergillosis where there is relative preservation of normal pulmonary structure without tissue infarction or airway obstruction that may compromise drug delivery and the antifungal effect. The pharmacodynamics in the early stages of infection are likely to be completely different from those later in the course of infection; secondly, in these studies there were no innate immunological effector cells or molecules, which are likely to provide additional antifungal activity; thirdly, we only studied a relatively small number of strains—there are likely to be at least some pharmacodynamic differences between strains and an interplay between drug activity and reduced fitness of strains carrying mutations; finally, we were unable to model the additional effect of the active metabolite hydroxyitraconazole. The intrapulmonary pharmacokinetics and pharmacodynamics of this metabolite has not been described and is worthy of further study.

Despite several limitations, this study provides a basis for the further investigation of innovative strategies to overcome triazole resistance. This study also provides an experimental basis for the further development of aerosolized antifungal therapy, which may be especially helpful for the prevention of IPA and/or the early treatment of this frequently devastating infection.
REFERENCES


Table 1. The minimum inhibitory concentrations (MICs) of the strains used in this study.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Itraconazole MIC (mg/L)</th>
<th>Itraconazole MIC (mg/L)</th>
<th>SFC MIC (mg/L)</th>
<th>SFC MIC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CLSI (range)</td>
<td>EUCAST (range)</td>
<td>CLSI (range)</td>
<td>EUCAST (range)</td>
</tr>
<tr>
<td>GFP (Wild-type)</td>
<td>0.25 (0.25-0.5)</td>
<td>0.5 (0.25-0.5)</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td>F/11628 (G138C)</td>
<td>&gt;8</td>
<td>&gt;8</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td>F/14532 (M220T)</td>
<td>&gt;8</td>
<td>&gt;8</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td>F/16216 (L98H)</td>
<td>&gt;8</td>
<td>&gt;8</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
</tbody>
</table>

The precise MICs could not be determined because of limitations in the solubility of itraconazole.
TABLE 2. The parameter estimates and their 95% confidence intervals describing the interaction of itraconazole administered to the alveolar compartment and 5FC administered to the endothelial compartment against *Aspergillus fumigatus* strain F/16216 (L98H).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Econ</td>
<td>6.35</td>
<td>5.50-7.20</td>
</tr>
<tr>
<td>$E_{50}$, itraconazole</td>
<td>8.77</td>
<td>6.04-11.49</td>
</tr>
<tr>
<td>$m$, itraconazole</td>
<td>1.13</td>
<td>0.72-1.53</td>
</tr>
<tr>
<td>$E_{50}$, 5FC</td>
<td>31.03</td>
<td>27.09-34.98</td>
</tr>
<tr>
<td>$m$, 5FC</td>
<td>2.31</td>
<td>2.11-2.51</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>$0.8 \times 10^{-7}$</td>
<td>-0.188-0.188</td>
</tr>
</tbody>
</table>

*a*Econ is the galactomannan concentration in the absence of any antifungal therapy; $E_{50}$, itraconazole is the initial concentration of itraconazole in the airway that produces half-maximal effect; $m$, itraconazole is the slope function for itraconazole; $E_{50}$, 5FC is the initial concentration of 5FC in the endothelial compartment that produces half-maximal effect; $m$, 5FC is the slope function for 5FC; $\alpha$ is the interaction term. Because the estimate for the interaction term ($\alpha$) is close to zero and its lower 95% confidence bound crosses zero, the interaction between airway administration of itraconazole and systemic administration of 5FC is additive.
FIG. 1. The pharmacokinetics of itraconazole in the in vitro model of the human alveolus. Itraconazole was administered to the endothelial compartment (systemic administration) and measured in the alveolar and endothelial compartments (left panels). Itraconazole was also administered to the alveolar compartment (airway administration) and concentrations subsequently measured in the alveolar and endothelial compartments (right panels).
FIG. 2. The pharmacodynamics of itraconazole against wild-type in the alveolar compartment (Panel A) and endothelial compartment (Panel B) following administration of drug to the endothelial compartment. For the alveolar compartment: Galactomannan=7.82-(5.06*[itra]^{3.10}/(1.25+[itra]^{3.10})); $r^2=0.78$. For the endothelial compartment: Galactomannan=7.74-(7.53*[itra]^{4.50}/(0.44+[itra]^{4.50})); $r^2=0.90$. Data are mean ± standard deviation.
FIG. 3. The pharmacodynamics of itraconazole against wild-type in the alveolar compartment (Panel A) and endothelial compartment (Panel B) following administration of drug to the alveolar compartment. For the alveolar compartment: Galactomannan=7.79-(5.25*itra^{2.29}/(2.00*itra^{2.29}+itra^{2.29})); $r^2=0.71$. For the endothelial compartment: Galactomannan=4.96-(4.49*itra^{4.91}/(0.65*itra^{4.91}+itra^{4.91})); $r^2=0.80$. Data are mean ± standard deviation.
FIG. 4. Exposure response relationships for itraconazole administered within the endothelial compartment against the mutants L98H, G138C and M220T. The upper and lower panels show the galactomannan concentrations in the alveolar and endothelial compartments, respectively. No exposure response relationships are evident.
FIG. 5. The pharmacodynamics of itraconazole against L98 mutant in the alveolar compartment (Panel A) and endothelial compartment (Panel B) following administration of drug to the alveolar compartment. No exposure response relationship is evident in the alveolar compartment. For the endothelial compartment: Galactomannan=5.40-(4.52*[itra]^{4.51}/(5.96^{4.51}+[itra]^{4.51})); \( r^2=0.50 \). Data are mean ± standard deviation.
FIG. 6. The pharmacodynamics of 5FC in the alveolar compartment (Panel A) and endothelial compartment (Panel B) following administration of drug to the endothelial compartment. Data are mean ± standard deviation. For the alveolar compartment: Galactomannan=8.10-(8.10*[5FC]^{4.78}/(100.20^{4.78}+[5FC]^{4.78})); r^2=0.94. For the endothelial compartment: Galactomannan=7.37-(7.22*[5FC]^{4.38}/(76.59^{4.38}+[5FC]^{4.38})); r^2=0.94.
FIG. 7. The fitted surface describing the effect of the combination of itraconazole administered to the alveolar compartment and 5FC administered to the endothelial compartment. The combination is additive. The raw data are not shown. The coefficient of determination ($r^2$) is 0.86.