Pharmacodynamics of isavuconazole in a Dynamic *In vitro* Model of Invasive Pulmonary Aspergillosis

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ABSTRACT

Background: Invasive pulmonary aspergillosis is a lethal infectious syndrome. Triazole resistance to isolates of *Aspergillus fumigatus* is of increasing clinical concern. Isavuconazole is a novel triazole agent in phase III clinical development. PK-PD analyses may provide decision support for safe and effective antifungal regimens and is an important component of setting *in vitro* susceptibility breakpoints.
Methods: A dynamic *in vitro* model of the human alveolus was used to describe the pharmacokinetics and pharmacodynamics of isavuconazole against two wild-type and two previously defined azole resistant isolates of *A. fumigatus*. A human-like concentration time profile for isavuconazole was generated. Minimum inhibitory concentrations were determined using CLSI and EUCAST methodologies. Galactomannan was used as a biomarker of fungal density. AUC:MIC target values were calculated using a population based mathematical model.
Results: Isolates with higher MICs required a higher AUC in order to achieve maximal suppression of galactomannan. The AUC:MIC target necessary to achieve 90% probability of galactomannan suppression was 11.40 for EUCAST methodology.
Conclusions: Data generated in this study may be bridged to humans to define susceptibility breakpoints for isavuconazole against *A. fumigatus* when clinical data is available.

BACKGROUND

The emergence of triazole resistance in *Aspergillus fumigatus* is of increasing clinical concern. An understanding of the pharmacokinetics and pharmacodynamics (PK/PD) of antifungal agents against *Aspergillus* spp. may assist clinicians in optimising the use of antifungal agents to improve clinical outcomes. Isavuconazole is a novel antifungal triazole agent in the latter phases of development. Isavuconazole exhibits potent antifungal activity *in vitro* against the majority of *Aspergillus fumigatus* isolates. PK-PD data obtained from studies of isavuconazole in mice may be difficult to bridge to humans because of substantial inter-species differences in clearance. Here, we use a previously described and validated dynamic in vitro model of the human alveolus, which enables human-like pharmacokinetics of isavuconazole to be simulated.

METHODS

ORGANISM AND PREPARATION OF INOCULUM

Isolates of *A.fumigatus* were recovered from -80°C stocks and sub-cultured onto potato dextrose agar for 5 days. Colonies were then washed with PBS and filtered. A conidial suspension of 1-3 x 10^4 was prepared using a haemocytometer and was confirmed by quantitative culture.

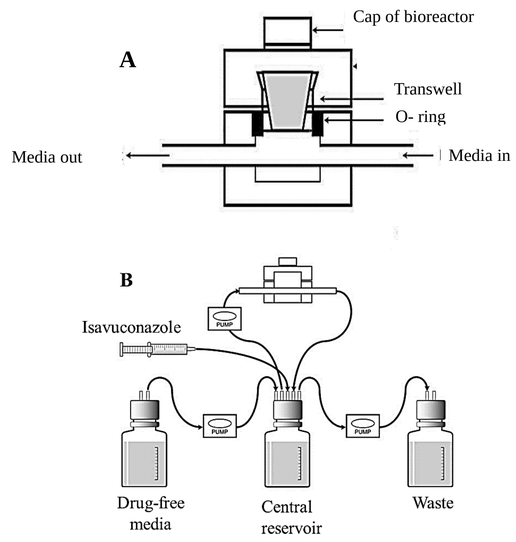
CONSTRUCTION OF CELLULAR BILAYER

A previously defined cell culture model of the human alveolus using 12-well cell culture plate inserts was used. The cellular bilayer consisted of human pulmonary arterial endothelial cells (HPAECs) and human alveolar epithelial cells (A549's).

ISAVUCONAZOLE PK-PD

The PK and PD of isavuconazole against *A. fumigatus* isolates were determined in four individual circuits. Isavuconazole dosages of 0mg, 0.1mg, 0.3mg, 0.5mg and 1.5mg were administered to the central compartment of each corresponding circuit. These dosages were used for all strains. Samples for both PK and PD were taken every 6 hours between 6-54 hours post inoculation.
Isavuconazole concentrations in media were measured using high performance liquid chromatography (HPLC) with a Shimadzu Prominence (Shimadzu).
Galactomannan (GM) was used as a quantitative biomarker in order to evaluate the antifungal efficacy of isavuconazole. Galactomannan levels were measured using a double-sandwich enzyme linked immunosorbent assay.

DYNAMIC MODEL OF THE HUMAN ALVEOLUS



MATHEMATICAL MODELLING

Equation 1 dX1/dt=B(1)-(SCL/Vc)*X1

Equation 2a dN/dt=Kgmax*(1-(N/POPMAX))*N

Equation 2b *(1-(X1/Vc)Hg/(X1/Vc)Hg+C50gHg))

- **Equation 1 describes the rate of change of isavuconazole concentrations in the circuit.**
- B(1) is the bolus input of isavuconazole; SCL is the clearance of isavuconazole; Vc is the volume of the circuit; N is the galactomannan concentration
- **Equation 2a describes fungal growth in the absence of isavuconazole**
- Kgmax is the maximal rate of growth; POPMAX is the theoretical maximal density within the circuit
- **Equation 2b describes the isavuconazole induced suppression of growth**
- Hg is the slope function for the suppression of growth
- C50g is the concentration of isavuconazole in the circuit where there is half-maximal suppression of growth.

RESULTS

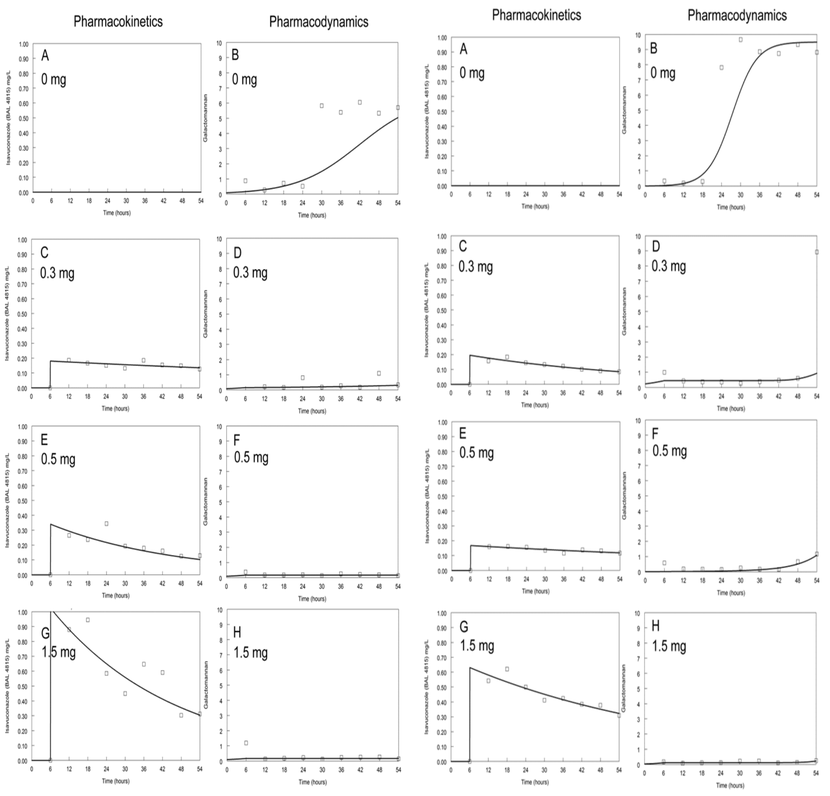


Figure 2. Pharmacokinetics and pharmacodynamics of isavuconazole against the wildtype. The modal minimum inhibitory concentration (MIC) of isavuconazole is 1 mg/L

Figure 3. Pharmacokinetics and pharmacodynamics of isavuconazole against NIH4215. The modal minimum inhibitory concentration (MIC) of isavuconazole is 1 mg/L

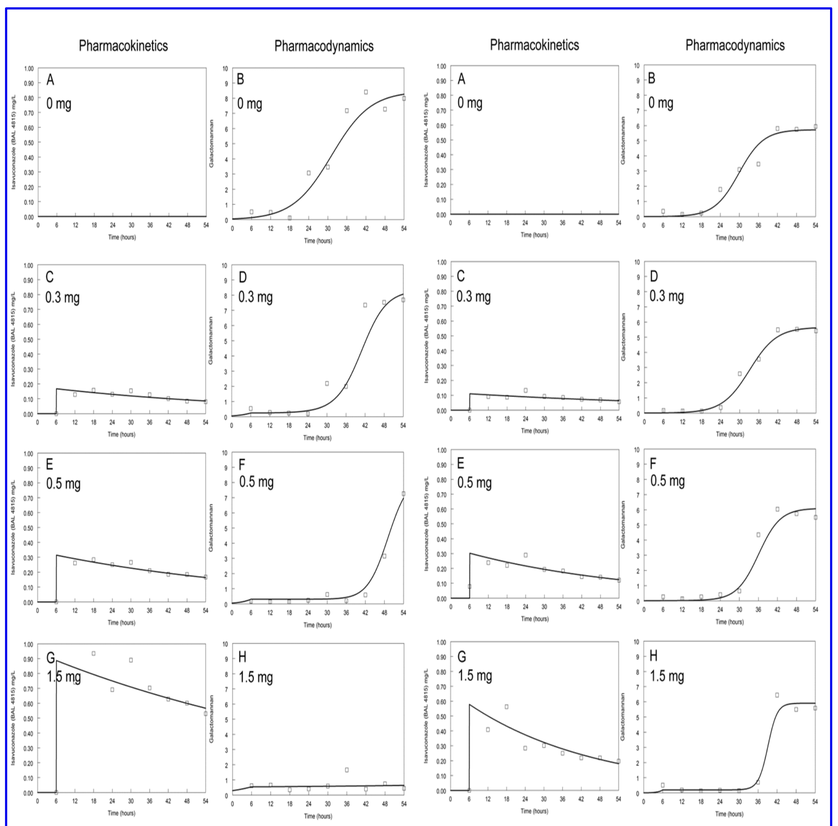


Figure 4. Pharmacokinetics and pharmacodynamics of isavuconazole against F/16216. The modal minimum inhibitory concentration (MIC) of isavuconazole is 4 mg/L

Figure 5. Pharmacokinetics and pharmacodynamics of isavuconazole against the F/11628. The modal minimum inhibitory concentration (MIC) of isavuconazole is 8 mg/L

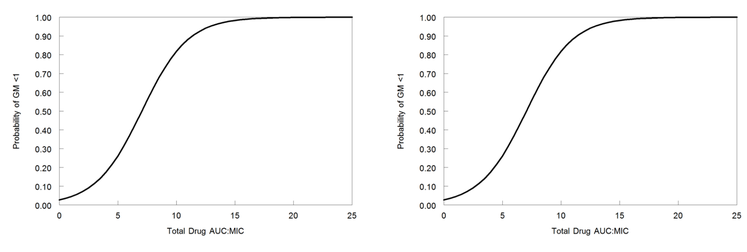


Figure 6 (a&b). The relationship between the AUC:MIC determined using EUCAST(a) and CLSI methodology (b) and the probability of galactomannan suppression. An AUC:MIC 11.40 is associated with a 90% probability of galactomannan suppression.

		EUCAST			CLSI		
Isolate	**Cyp51A mutation**	**Mode**	**Range**	**Mean**	**Mode**	**Range**	**Mean**
Wildtype	Wild-type	1	0.5-2.0	1	1	0.5-1	0.81
4215	Wild-type	1	0.5-2.0	1	1	1-4	1.62
16216	L98H	2	2-4	2.64	4	1-4	2.63
11628	G138C	4	4-8	4.92	4	1-8	2.82

Table 1. Minimum inhibitory concentrations were determined in 10 independent experiments. Means displayed above are geometric means.

CONCLUSIONS

- Isavuconazole causes a concentration-dependent reduction in galactomannan concentrations that are liberated from *A. fumigatus*.
- The MIC and the genotype account for a considerable portion of pharmacodynamic differences that are observed between strains.
- An AUC:MIC target of 11.40 is associated with near maximal antifungal activity in this model of early invasive pulmonary aspergillosis.
- These results should be considered in conjunction with results from other pharmacodynamic models and data from other investigators/ laboratories.
- Data generated in this study may be bridged to humans to define susceptibility breakpoints for isavuconazole against *A. fumigatus* when clinical data is available.

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