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Pharmacodynamics of the active moiety APX001A in human pulmonary and nasal *in vitro* models of invasive aspergillosis

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ABSTRACT

Background: Invasive aspergillosis is a common and frequently lethal disease. Treatment is restricted to few antifungal compounds and resistance is an emerging public health problem. The development of new antifungal compounds is essential. APX001A is the active moiety of APX001 prodrug, a novel first-in-class cell wall active antifungal agent that has been demonstrated a broad-spectrum activity *in vitro* against *Candida* and *Aspergillus*, including strains resistant to currently antifungal drugs. The aim of this study was to evaluate the in vitro pharmacodynamics of APX001A against the most common pathogenic species, *A. fumigatus* and *A. flavus*.
Material/methods: In order to evaluate the efficacy of APX001A against *Aspergillus fumigatus*, a well-characterised *in vitro* model of human pulmonary invasive aspergillosis was used. Briefly, a bilayer of human pulmonary artery endothelial cells and human alveolar epithelial cells were cultured on a semipermeable polyester membrane. The bilayer was inoculated with *A. fumigatus* conidial suspension on the alveolar surface. Three strains were used: a triazole wild type (NIH4215) and two with different *CypA* amino acid substitutions (L98H and G138C) that confer resistance to triazole antifungal agents. An additional *in vitro* model that uses human nasal epithelial cells for the epithelial layer was developed to study the pharmacodynamics of APX001A against *Aspergillus flavus*. Two *A.flavus* clinical strains were used (LEMI764 and LEMI1024). Infected cell bilayers were then exposed to seven different APX001A concentrations ranging from 0.015 to 1mg/L for 48 hours. Media from the endothelial surface, as well as epithelial surface lavage, were analysed.
Galactomannan (GM) was used as the model readout. Experiments were performed in triplicate.
Results: Related to *A. fumigatus* pulmonary infection, 0.25 mg/L of APX001A was able to suppress GM index (<1) in the endothelial compartment infected with NIH4215. For L98H and G138C strains only 0.03 mg/L and 0.125 mg/L was required to suppress GM, respectively. For the *A. flavus* nasal infection the lowest concentration tested, 0.015mg/L, was able to suppress GM index in the endothelial surface of the model for both strains tested.
Conclusions: These findings suggest that APX001A is a potent antifungal agent against *A. fumigatus* and *A. flavus*. APX001A induces a concentration-dependent decline in galactomannan. Further *in vivo* studies are required to fully characterise the pharmacodynamics of APX001A.

INTRODUCTION AND PURPOSE

Aspergillus fumigatus and *Aspergillus flavus* are the most frequent species causing invasive aspergillosis accompanying generally inferior and superior respiratory tract, respectively. Both invasive pulmonary aspergillosis and invasive rhinosinusitis caused by *Aspergillus* are normally lethal, with mortality rates from 40-80% worldwide. Few antifungal classes are commercial available and since 2002 voriconazole has been recommended as first choice therapy for invasive aspergillosis . Nevertheless, azole resistance has become an emergent health problem in many countries. There is an emergence of cryptical species intrinsically resistant to available antifungal compounds as human pathogen as well as acquired resistance mechanisms by *A. fumigatus* and *A. flavus*. Therefore, the development of new antifungal compounds with new mechanisms of action is crucial. APX001A is the active moiety of APX001 prodrug, a novel and promising first-in-class compound. APX001A acts by inhibiting inositol acyltransferase, an early step of Glycosylphosphatidylinositol (GPI)-anchored proteins maturation. *In vitro* susceptibility tests have demonstrated a broad-spectrum activity of APX001A against *Candida* and *Aspergillus*, including strains resistant to currently antifungal drugs. The aim of this study was to evaluate the *in vitro* pharmacodynamics of APX001A against the most common pathogenic species, *A. fumigatus* and *A. flavus* in two different static models of infection.

METHODS

In order to evaluate the efficacy of APX001A against *Aspergillus fumigatus*, a well-characterised *in vitro* model of human pulmonary invasive aspergillosis was used.
Briefly, a bilayer of human pulmonary artery endothelial cells (HPAECs) and human alveolar epithelial cells (A549s) were cultured on a semipermeable polyester membrane. The bilayer was inoculated with *A. fumigatus* conidial suspension on the alveolar surface. Three strains were selected and sub-cultured onto SAB plates at 37˚C for 4-7 days: a triazole wild type (NIH4215) and two with different *CypA* amino acid substitutions (L98H and G138C) that confer resistance to triazole antifungal agents.
An additional *in vitro* model that uses human nasal epithelial cells (HNECs) for the epithelial layer was developed to study the pharmacodynamics of APX001A against *Aspergillus flavus*. Two *A.flavus* strains were selected (LEMI764 and LEMI1024) from patients with invasive aspergillosis.
Both pulmonary and nasal cellular bilayers were infected by *A.fumigatus* or *A. flavus* inoculum, respectively with a conidial suspension of 1 x 10^4. The conidial suspension was prepared using a haemocytometer and was confirmed by quantitative culture.
Infected cell bilayers were then exposed to seven different APX001A concentrations ranging from 0.015 to 1mg/L for 48 hours (Figure 1).
Media from the endothelial surface, as well as epithelial surface lavage, were analysed. Galactomannan (GM) was used as the model readout for pharmacodynamics evaluation. Experiments were performed in triplicate.

RESULTS

FIGURE 1. STATIC MODEL OF INVASIVE ASPERGILLOSIS

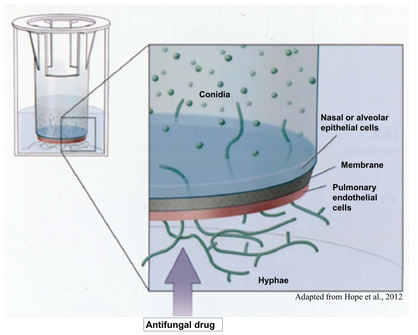
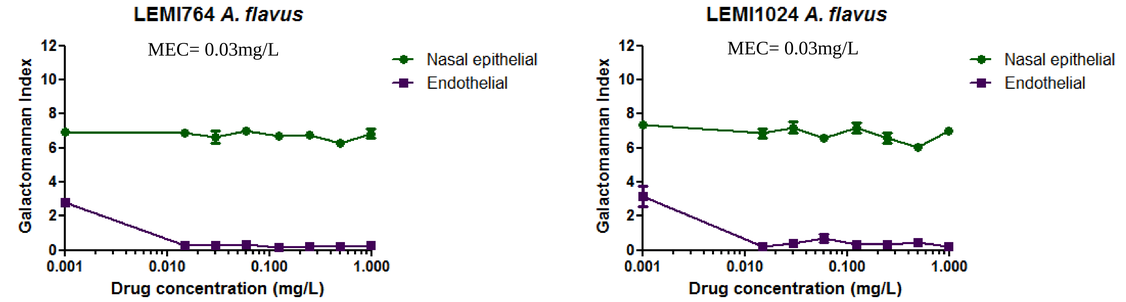
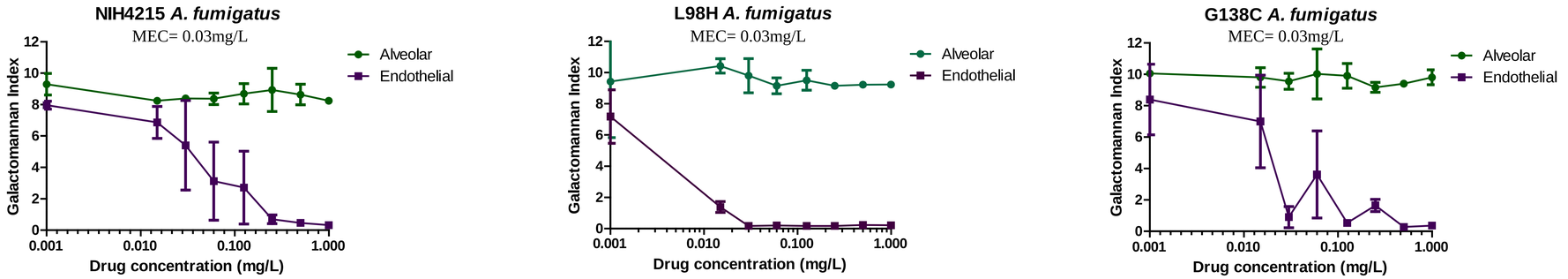


FIGURE 2. APX001A EXPOSURE RESPONSE IN *A. Flavus IN VITRO* RHINOSINUSITIS MODEL



MEC: Minimum effective concentration, according to CLSI M38-A2 methodology

FIGURE 3. APX001A EXPOSURE RESPONSE IN A. *fumigatus IN VITRO* INVASIVE PULMONARY MODEL



MEC: Minimum effective concentration, according to CLSI M38-A2 methodology

CONCLUSIONS

1- For the *in vitro* static models APX001A is a potent antifungal agent against *A. fumigatus* and *A. flavus*
2- APX001A induces a concentration-dependent decline in galactomannan.
   a. For the *A. fumigatus* pulmonary infection, 0.25 mg/L APX001A suppressed GM index (<1) in the endothelial compartment infected with NIH4215. For the L98H and G138C strains, 0.03 mg/L and 0.125 mg/L suppressed GM, respectively.
   b. For the *A. flavus* nasal infection the lowest concentration tested, 0.015mg/L, was able to suppress GM index in the endothelial surface of the model for both strains tested.
3- Further *in vivo* studies are required to fully characterise the pharmacodynamics of APX001A.

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