“Repurposing FDA-approved Ebselen and Auranofin as anti-fungal drugs: “teaching old drugs new tricks”

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Abstract

Objectives: Fungal infections represent an increasing threat to an expanding population of at risk patients; but the impact of these diseases on human health is not widely appreciated. The mortality associated with some of these infections remain unacceptably high. Because fungi are eukaryotic, there is a paucity of selective targets that can be exploited for antifungal drug development. As a result, the current antifungal armamentarium is very limited. Furthermore, the antifungal drug pipeline is mostly dry. There is no doubt that novel antifungals and new approaches to develop them are urgently warranted. Unlike the costly and time consuming process of de novo drug discovery, drug repurposing is a novel method to reduce the time, cost and risk associated with drug innovation. **Methods:** The present studies build upon the recent discovery by our group of the potent antifungal activity of FDA-approved drug Auranofin, which we originally identified in high content screenings of the Prestwick library against *Candida albicans* biofilms. MICs were determined in accordance with the CLSI M27-A3 (for yeast) and M38-A2 (for filamentous fungi) reference standards for antifungal susceptibility testing. We also performed *in-vivo* evaluation of these drugs in the murine models of systemic cryptococcosis and hematogenously disseminated candidiasis. **Results:** Screening the same library against other pathogenic fungi resulted in the identification of Ebselen as a leading compound displaying potent antifungal activity against different fungi. We observed increased survival of mice in groups treated with Auranofin and Ebselen compared to mock treated groups. Both drugs are organometallics and have many advantageous qualities including oral bioavailability, potent microbicidal activity in a clinical range and very low frequency of resistance. **Conclusion:** In conclusion, repositioning old drugs can significantly decrease the time and effort that it takes to develop novel antifungal therapeutics, which represents a pressing and unmet clinical need due to the devastating nature of fungal infections. Thus, the present study seeks to provide further valuable evidence that both drugs have significant promise to be repositioned as novel antifungals for the treatment of fungal infections.

Materials & Methods

Determining the inhibitory activity and spectrum : All the *in vitro* susceptibility testing experiments was performed following CLSI reference methods, including CLSI M27-A3 (for yeasts) and M38-A2 (for filamentous fungi). Using these broth microdilution techniques we have determined the *in vitro* activity of Auranofin and Ebselen against *Cryptococcus spp* and *Candida spp*. Combination of lead molecules with standard antifungal drugs was performed by checker-board analysis.

Efficacy of Auranofin and Ebselen in clinically-relevant murine models of Cryptococcosis: One group of animals was served as control (treated with vehicle only), and at the same time different groups of animals was treated with Auranofin and/or Ebselen, injected intraperitoneally, once daily, starting one day post-infection. A standard dose, based on *in vitro* results and prior data from animal models for their other indications, was 5 mg/kg for Auranofin and Ebselen.

Results

Figure.1 Results from primary screenings identified Auranofin and Ebselen as two of the major hits with antifungal activity against both *C. albicans* and *C. neoformans*

Fig. 2 Survival Analysis: Mice were treated via the intra-peritoneal route with Ebselen, Auranofin and Mock (vehicle control) rested for 1 day, and then challenged intravenously with *Cryptococcus neoformans* H99 10⁴ CFU. Survival was monitored twice daily and mice that appeared moribund or not maintaining normal habits (grooming) were sacrificed

Conclusions & Future Directions

- Drug repurposing is a novel method to reduce the time, cost and risk associated with drug innovation. We found potent antifungal activity of FDA-approved drugs Auranofin and Ebselen, against *Cryptococcus neoformans* and *Candida spp*. including resistant *C. auris*. *In vivo* analysis also shows significant increase in animals treated with Ebselen and Auranofin compared to mock treated animals in murine model of disseminated cryptococcosis.
- Further studies is needed to enhance the efficacy by determining the optimum dose of these two drugs. Also fungal burden and histopathology analysis is required.

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Drugs	<i>C. neoformans</i> (serotype A) H99	<i>C. deuterogattii</i> (serotype B) R265	<i>C. bacillisporus</i> (serotype C) WSA87	<i>C. deneoformans</i> (serotype D) R4247
Ebselen	0.39µg/ml	0.78µg/ml	0.39µg/ml	0.39µg/ml
Auranofin	0.19µg/ml	0.39µg/ml	0.19µg/ml	0.19µg/ml

Table 1. MICs of Auranofin and Ebselen against *Cryptococcus spp*.

Strains	Combination	FICI
H99	Auranofin+Fluconazole	0.1406
WSA87	Auranofin +Caspofungin	0.3125
WSA87	Ebselen+ Caspofungin	0.0625
R265	Auranofin + Caspofungin	0.3125
R265	Ebselen+Fluconazole	0.0781
R4247	Auranofin + Caspofungin	0.3125

Table 2. Synerstic effect of the compounds with standard antifungal drugs.

<i>Candida auris</i> strain	Calculated IC50:Inhibition of Planktonic Growth (g/ml)	Calculated IC50:Inhibition of Biofilm Formation (µg/ml)
0381	0.2521	6.067
0382	1.94	8.98
0383	0.4198	9.781
0384	0.1673	7.432
0385	0.9163	9.164
0386	0.5008	9.425
0387	2.283	9.006
0388	0.1643	8.821
0389	1.217	5.864
0390	0.3561	8.05

Table 3. Calculated IC50 of Ebselen for Inhibition of Planktonic Growth and Inhibition of Biofilm Formation: *C. auris* 0381-0390