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Toward improved anti-cryptococcal drugs: Novel molecules and repurposed drugs

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ABSTRACT

Cryptococcosis is one of the most important fungal infections of humans. It primarily, but not exclusively, afflicts people with compromised immune function. Cryptococcosis is most commonly caused by *Cryptococcus neoformans* var. *grubii* with *C. neoformans* var. *neoformans* and *C. gatti* also contributing to the disease. Cryptococcosis is primarily manifested as meningoencephalitis although pneumonia occurs frequently as well. Globally, the burden of disease is highest among those living with HIV/AIDS and is one of the most common causes of death in this patient population. Cryptococcal meningitisis almost invariably fatal if untreated. The current gold standard therapy is amphotericin B combined with 5-flucytosine. Unfortunately, this therapy has significant toxicity and is not widely available in resource-limited regions. Fluconazole, which is associated with poorer outcomes, is frequently as an alternative. Here, I present the characteristics of an ideal anti-cryptococcal agent and review recent progress toward identifying both novel and repurposed drugs as potential new therapies.

0. Introduction

Cryptococcosis is one of the most important, life-threatening fungal infections of humans (Chayakulkeeree and Perfect, 2006). The vast majority of cryptococcosis manifests as meningoencephalitis in immunocompromised individuals and is due to one of three primary species of *Cryptococcus*: *C. neoformans* var. *grubii*; *C. neoformans* var. *neoformans*; and *C. gattii*. The global significance of cryptococcosis is due in large part to its prevalence as a cause of meningoencephalitis in people living with HIV–AIDS. In some regions of the world, it has surpassed tuberculosis as an infectious cause of HIV–AIDS-associated mortality (Park et al., 2009). However, it also affects people with other immune-compromising conditions. Furthermore, an ongoing outbreak of disease due to *C. gatti* in the Pacific Northwest region of North America has primarily affected individuals with no apparent alteration in immune function (Rolston, 2013). Here, I review the current therapeutic options for cryptococcosis; the limitations and un-met clinical needs of these therapies; the challenges to developing new agents; and recent progress in the identification of new small molecules with anti-cryptococcal activity.

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1. Current anti-cryptococcal drugs and regimens

The gold standard antifungal regimen for the treatment of cryptococcal meningoencephalitis is a combination of the polyene amphotericin B with the uracil derivative 5-fluoro-uracil (Fig. 1). Although this regimen has been employed for many years, only recently has a clinical trial demonstrated that the combination is more effective than amphotericin B alone (Day et al., 2013); 5-fluoro-uracil is not amenable to monotherapy because of the rapid development of resistance. Unfortunately, amphotericin B has significant nephrotoxicity and requires intravenous administration, limiting its use in regions without strong medical infrastructure (Sloan et al., 2009). 5-Fluorouracil is not widely available outside of resource rich areas. In addition, 5-fluorouracil has hematologic toxicities. The toxicity associated with amphotericin B and 5-flucytosine regimen is presumably one of the reasons why only 37% of patients in a major medical center in the US receive the gold-standard therapy (Perfect, 2013).

In resource-limited regions, fluconazole is the most commonly used alternative to amphotericin B (Sloan et al., 2009). Although fluconazole inhibits the growth of *Cryptococcus* at low drug concentrations, a number of clinical studies have shown that it is less effective than amphotericin B-based therapy (Sloan et al., 2009). The most likely reason for this difference in outcome is that amphotericin B is fungicidal toward *Cryptococcus* while fluconazole

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is fungistatic. The clearance of organisms from the cerebrospinal fluid, termed the early fungicidal activity (EFA), has been shown to correlate with outcome (Bicanic et al., 2009). Compared to amphotericin B, fluconazole has a very poor EFA even at high doses.

Taken together, these considerations clearly indicate that new broadly available fungicidal anti-cryptococcal drugs would be enormously beneficial. The current standard of care is based on medications that are over 50 years-old and leave much to be desired for patients in both resource-rich and resource-limited regions of the world (Butts and Krysan, 2012). Unfortunately, the development of new antifungal and antimicrobial therapies has slowed dramatically in recent years because pharmaceutical companies have left the arena. The newest class of antifungal drugs introduced into clinical practice was the echinocandins (Roemer and Krysan, 2014). The class was first identified in the early 1970s, was brought to market 30 years later in the early 2000s, and has now been in use for over 10 years. No new classes have progressed in clinical development in the last 10 years. Unfortunately, the echinocandins have no clinically useful activity against *Cryptococcus* (Perfect et al., 2010). In the sections below, I will outline some characteristics that an ideal anti-cryptococcal drug will have; review recent developments in the identification of new classes of molecules with anti-cryptococcal activity; and discuss new approaches to screening for molecules with anti-cryptococcal activity.

2. Characteristics of an ideal anti-cryptoccocal drug

In addition to having antifungal activity toward Cryptococcus, an effective anti-cryptococcal agent must have a number of microbiological and pharmacological properties that are not necessarily required for the treatment of other invasive fungal infections (Fig. 1). First, as discussed above, fungicidal activity has been shown to be associated with improved outcome for cryptococcal meninigitis as compared with drugs with fungistatic activity (Bicanic et al., 2009). Currently, the only clinically used drug with fungicidal activity toward *Cryptococcus* is amphotericin B.

Second, the most common manifestation of cryptococcosis is meningoencephalitis (Chayakulkeeree and Perfect, 2006) and, therefore, any effective therapy must penetrate the blood–brain barrier of the central nervous system (CNS). Not all drugs penetrate the central nervous system. Much has been learned recently about the characteristics of drugs that reach high levels in the CNS. However, it remains difficulty to predict exactly which drugs will be useful in the treatment of CNS disease.

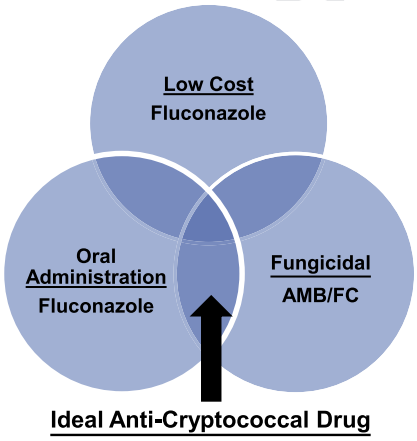

Fig. 1. Characteristics of an ideal anti-cryptococcal drug.

Third, the largest burden of cryptococcal disease is associated with regions of the world with high rates of HIV infection (Park et al., 2009). Many of these regions have limited medical resources and infrastructures (Sloan et al., 2009). Therefore, new therapies need to be low cost and logistically simple to obtain and administer. As such, an orally bioavailable drug is essentially an imperative characteristic for a new cryptococcal agent with any hope of addressing the global burden of disease. The oral bioavailability of fluconazole is one of the main features driving its use as the alternative therapy in resource-limited regions (Sloan et al., 2009) and, conversely, a significant limitation to the widespread use of amphotericin B is the need for hospitalization and intravenous administration. The cost and availability of the drug will also have to be considered in this context. From this point of view, it seems reasonable to consider the example of drug development for parasitic diseases or other so-called "neglected infectious diseases". In this case, the logistical and economical aspects of candidate therapies play an important role in every step of the drug development process. A similar paradigm will be required if we are to develop new therapies that will address the un-met clinical needs of treating cryptococcal disease world-wide.

As highlighted in Fig. 1, none of the current drugs used to treat cryptococcosis meets all of the ideal criteria for an anti-cryptococcal agent. Amphotericin B is intravenous and requires laboratory monitoring for potential toxicity; fluconazole is fungistatic and not fungicidal; and 5-flucytosine is only adjunctive and also requires laboratory monitoring of potential toxicity. Finally, only fluconazole is widely available in resource-limited regions with high burden of disease and it is the only therapy that is administered orally. Clearly, a drug that met all of the characteristics of an ideal cryptococcal therapy would be of huge clinical importance for global health.

3. Recently reported novel small molecules with anti-cryptococcal activity

Relatively few novel chemical scaffolds with anti-cryptococcal activity have been reported in recent years. For example, two new structural classes of fungal cell wall biosynthesis inhibitors (E1201 and D75-4590) are active against *Candida* spp. and *Aspergillus* but have no activity against *C. neoformans* (Hata et al., 2011; Tsukahara et al., 2003) coupled with the low activity of the echinocandins against *C. neoformans*, a cell wall-targeted anti-cryptococcal molecule remains an elusive but potentially valuable type of molecule.

Two new classes of molecules with anti-cryptoccocal activity have been reported recently (Fig. 2A and B): the 1,2-benzisothiazolinones (BTZ; Dou et al., 2011) and a set of arylamidines (T-2307, Mitsuyama et al., 2008) derivatives related to the anti-parasitic and anti-pneumocystis drug, pentamidine. Both scaffolds have broad spectrum activity against *Candida* spp. and *Aspergillus* spp. Interestingly, both molecules interfere with mitochondrial respiration as part of their mode of action (Alex et al., 2012; Shibata et al., 2012). For example, both BZT and T2307 lead to decreased mitochondrial membrane potential. The MIC of T-2307 is much lower when cells are grown in glycerol (Shibata et al., 2012), a substrate that requires mitochondrial respiration to support growth. Although drugs targeting a conserved structure such as the mitochondria may not seem attractive at first, the BZT compounds have good cytotoxicity profiles in vitro (Alex et al., 2012) and T-2307 appears to be selectivity active against fungal mitochondria (Shibata et al., 2012). Consistent with the low mammalian toxicity, T-2307 has been shown to be effective in the treatment of disseminated cryptococcosis in mice (Mitsuyama et al., 2008). Furthermore, it is certainly possible that fungal specific drug targets

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