Fungal pathogenesis

https://doi.org/10.1038/s41564-023-01595-5

Metabolic sensing tips the balance of drug tolerance in fungal meningitis

Austin Mottola, Johannes Hartl, Markus Ralser & Judith Berman

Sensing of brain glucose by the fungal pathogen *Cryptococcus neoformans* alters lipid metabolism and membrane composition in the fungus, rendering it drug tolerant.

Human fungal pathogens are underappreciated killers. Invasive infections with *Cryptococcus* are estimated to cause over 1 million life-threatening infections, largely meningitis, per year worldwide[1]. Contributing factors include weakened immune systems in immunocompromised patients and the difficulty of delivering antifungal drugs to sites of infection. Other factors such as host–pathogen interactions and how they affect drug responses remain unclear. Writing in *Nature Microbiology*, Chen, Tian, Zhang and colleagues asked whether host-derived metabolites can enable *Cryptococcus neoformans* to better withstand exposure to amphotericin B (AmB)[2], the antifungal primarily used to treat cryptococcosis (infection with *C. neoformans*). Surprisingly, they found that glucose, which is replete in the brain but rare at other sites of *Cryptococcus* infection, causes changes in the ratio of fungal cell membrane components. These changes protect the fungus from AmB treatment, but also expose new vulnerabilities that adjuvant drugs, given together with AmB, may be able to target.

Currently, the preferred drug for treating cryptococcal meningitis is AmB[3], which celebrated the 70th anniversary of its discovery last year. Yet mortality rates of cryptococcal meningitis can reach 70%, despite very low rates of AmB resistance[1]. AmB is a double-edged sword — while resistance is rarely seen, nephrotoxicity makes it difficult for most patients to complete treatment regimes. Improvements in drug delivery were made in the intervening years — specifically, a liposomal formulation developed in the 1990s that enhances solubility and delivery to target sites — but toxicity remains an issue. Recently, a modification of AmB that reduces toxicity was reported[4], providing an exciting opportunity for improved treatment.

Another reason why antifungals can fail is drug tolerance, a phenomenon of particular importance to fungal infections. Drug tolerance differs from drug resistance and is a type of phenotypic heterogeneity — a non-genetic, physiological drug response[5]. For microbicidal drugs such as AmB, antimicrobial tolerance is defined as the ability of a subpopulation of cells to withstand prolonged drug exposure at concentrations that kill the majority of the population[6]. Tolerance is increasingly associated with the treatment failure of fungistatic drugs used to treat candidiasis (*Candida* infections)[7–10]. However, because it is a sub-population phenomenon, and is affected by environmental factors such as pH and temperature[7,11], tolerance is not routinely tested in clinical assays and does not inform treatment decisions. In this study, the authors provide mechanistic insights into how *C. neoformans*, which is phylogenetically distant from *Candida* species, can be tolerant to AmB, a fungicidal drug.

By screening metabolites for their effect on the survival of *C. neoformans* exposed to AmB, the authors found that AmB tolerance increases in high-glucose media. This requires signalling through Mig1, an activator of glucose repression, a pathway common to many microorganisms that ensures preferential glucose uptake. However, higher glucose concentration did not make *C. neoformans* more resistant to the drug: the minimum drug concentration required to halt growth was not altered by glucose catabolism. Rather, glucose sensing increased the ability of the fungus to tolerate the fungicidal drug.

Transcriptomics then guided the authors to the lipidome. A series of targeted as well as exploratory investigations eventually revealed a compelling and novel mechanistic explanation: Mig1 activation not only affects flux through glucose repression, it also reduces the amount of membrane ergosterol and increases the amount of membrane sphingolipids. Ergosterol is the membrane sterol targeted by AmB (Fig. 1a), and thus is required for its efficacy. Moreover, the reduced amounts of ergosterol and the compensatory increase in sphingolipids associated with Mig1 activity make *C. neoformans* tolerant to AmB. In particular, inositolphosphorylceramide — an anionic sphingolipid — binds ergosterol more tightly than AmB, effectively competing with the drug. Notably, glucose signalling increases the proportion of cells that can survive AmB exposure; this effect could not have been observed in standard clinical susceptibility tests. Thus, Mig1-mediated glucose signalling tilts the scale in favour of survival under drug stress (Fig. 1b).

Does this new understanding of the interplay between glucose and drug response have relevance in vivo? To test the hypothesis that the protective effect of glucose allows more fungal cells to survive treatment, the authors infected mice with *C. neoformans* with or without a copy of the *MIG1* gene. Upon AmB treatment, mice infected with mutants lacking *MIG1*, which did not exhibit glucose-induced AmB tolerance, survived better and had less *C. neoformans* in their brain tissue than mice infected with the wild-type parent strain. AmB was also more effective at eliminating *C. neoformans* growing in glucose-depleted human cerebrospinal fluid. Thus, the chemical environment of the host encountered by infecting fungi may be an underappreciated source of variability in drug efficacy and treatment failure.

The authors then asked whether this new understanding of the interplay between glucose and drug response would have implications for the treatment of fungal meningitis. By combining AmB with aureobasidin A (AbA), which inhibits sphingolipid biosynthesis, the authors tested the idea that inhibiting the production of membrane sphingolipids, which are produced in greater quantities in the presence of glucose, would compete with AmB (Fig. 1b). Importantly, combining these two drugs reduced AmB tolerance in vitro and improved the survival of infected mice more so than AmB with flucytosine, the currently recommended antifungal drug combination for treating fungal meningitis.

Drug combinations are more difficult to translate to patients than monotherapies, yet they remain an important strategy for the

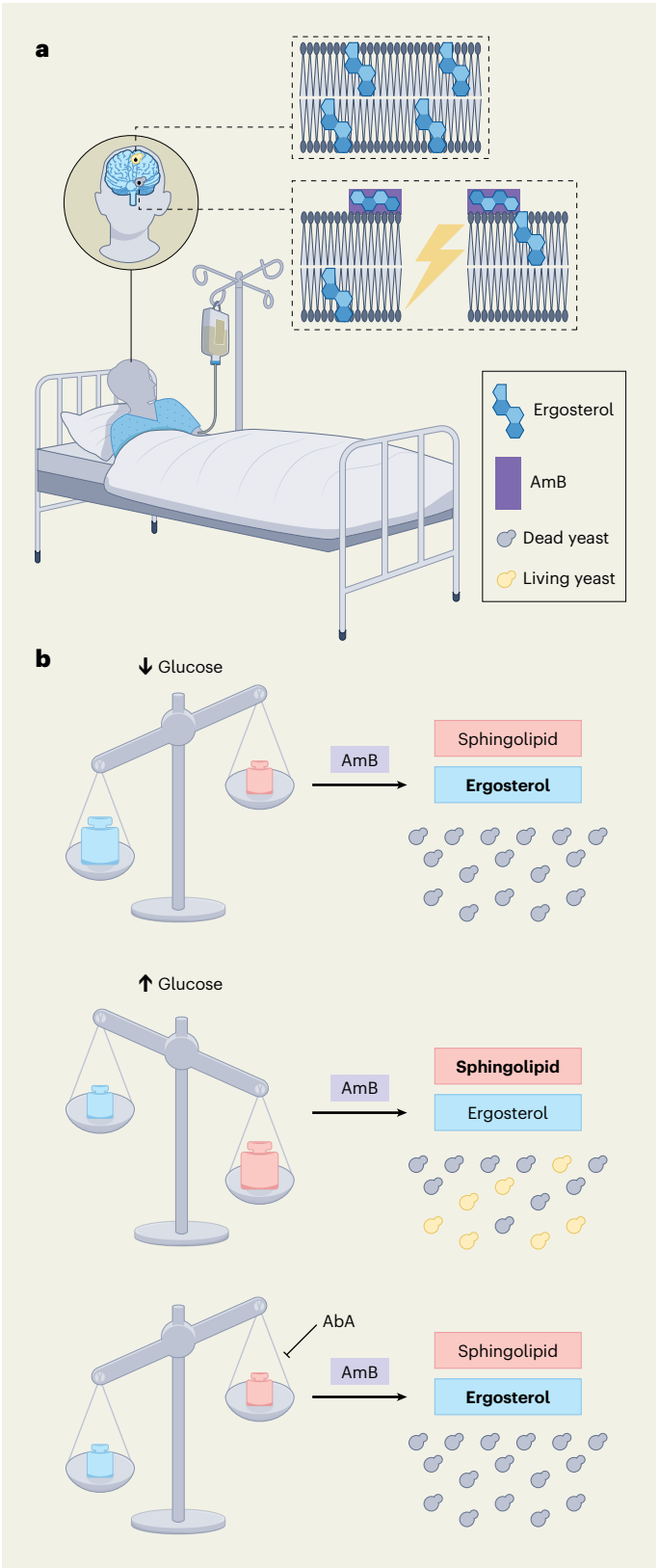

Fig. 1 | Glucose sensing alters *Cryptococcus* membrane composition leading to AmB tolerance. a, Mechanism of the action of AmB. AmB associates with ergosterol in the fungal membrane, sequestering it and consequently inducing pore formation, depolarization and cell death. **b**, When glucose is limited, AmB efficiently kills cryptococcal cells (top). However, heightened glucose levels activate glucose repression through Mig1 in *Cryptococcus*. Mig1 activation also changes the balance of membrane lipids so that sphingolipids associate with and protect ergosterol from AmB, allowing some cells to survive (middle). Inhibition of sphingolipid biosynthesis by AbA undermines this effect, making cells susceptible once again to AmB (bottom).

treatment of fungal infections. The high degree of genetic similarity between fungi and humans makes finding effective and selective antifungal drug targets extremely challenging. Consequently, only three different mode-of-action classes of antifungal drugs are clinically approved for invasive fungal infections[12]. Fortunately, promising novel antifungals for treating cryptococcal meningitis, such as fosmanogepix, are in the pipeline and in clinical evaluation[13]. Nonetheless, this limited arsenal drives the need for strategies that improve the efficacy of the existing antifungals.

This study makes a compelling case that highlights that in addition to drug resistance, the role of both drug tolerance and its metabolism dependence are important considerations in the treatment of fungal infections. To this end, exploiting fungal dependencies, either pharmacologically or by manipulating metabolite levels in the fungal microenvironment – for example, via diet interventions – may provide new routes to improving antifungal treatment efficacy and ultimately disease outcome.

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Published online: 5 February 2024

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Competing interests

M.R. is a founder and a shareholder of Elitpca Ltd. All other authors declare no competing interests.