Involvement of iron metabolism in fluconazole susceptibility in *Saccharomyces cerevisiae* and *Candida* species

Liesbeth Demuyser, E. Swinnen, A. Fiori, B. Herrera-Malaver, K. Verstrepen and P. Van Dijck

VIB center for Microbiology and KU Leuven Department of Biology, Belgium

KU LEUVEN

Why worry about fungal infections?

Fungal infections pose a **significant threat** to the health of humans. Some of these infections are superficial and impose a mild form of inconvenience, while others are invasive, causing severe disease and, potentially, death. Once an invasive infection is established, the likelihood of survival for the patient rarely exceeds 50%¹.

Our conclusion

From our experimental evidence, we hypothesize that **iron metabolism** plays a central role in regulating fungal susceptibility to fluconazole. **Fe-S cluster formation** as well as **activation of the iron regulon** lead to protection of the cell from fluconazole by retaining higher amounts of ergosterol².

Fungal infections pose a significant threat

Iron metabolism aids in protection against fluconazole

Iron

Fluconazole, most-used drug, yet ineffective

Combination therapy to extinct *Candida* infection

What is the problem?

The number of **drugs** available against fungal infections is **limited**, and those that are commonly used often suffer from being **fungistatic** rather than fungicidal. The azoles, with **fluconazole** (flu) being the most studied, comprise one of these commonly used, fungistatic classes of antifungals. The azoles target the ergosterol pathway, more particularly, the lanosterol 14 α -demethylase (Erg11).

What is our aim?

By understanding how *S. cerevisiae* and pathogenic fungi react to and cope with the presence of clinically-used drugs, we can learn **how to potentiate these drugs** and, as such, increase the effectiveness of treatment.

The experimental evidence

Fe-S metabolism influences susceptibility to fluconazole

We performed a screening of the model yeast *S. cerevisiae* transformed with multicopy plasmids, containing parts of its genomic library. Using sensitized screening conditions³, we isolated **Mge1** as a dosage-dependent **suppressor of susceptibility to fluconazole**. Mge1 is a cochaperone for the Hsp70 chaperone **Ssq1**, which is involved in the **Fe-S cluster biosynthesis pathway**⁴. We tested the MIC_{flu} of the *ssq1* Δ strain and found it to be significantly lower than the BY4742 wild type strain.

Strain	Etest MIC _{flu} (μ g/ml) - EV	Etest MIC _{flu} (μ g/ml) - <i>MGE1</i> ox
BY4742	6-8	24-32
<i>Scssq1</i> Δ	2-4	1-1.5
<i>Scaft1</i> Δ	4-6	4-6
<i>Scaft2</i> Δ	4-6	32-48
<i>Scfra1</i> Δ	6-8	/
HTL (<i>C. glabrata</i>)	8	24

MGE1 ox increases the level of ergosterol under fluconazole

We performed GC-MS analysis of the sterols isolated from both the control and the mutant. The **decrease in ergosterol upon fluconazole addition, is reduced when *MGE1* is overexpressed** (fig. 1). We, furthermore, show that the increase in lanosterol observed in the control upon fluconazole addition, is diminished in our mutant strain. The same is observed for 14-methylfecosterol and the toxic 14-methylergosta-8,24(28)-dien-3 β ,6 α -diol. This leads us to hypothesize that **Mge1 diverts the flux in the sterol synthesis pathway away from the toxic sterol and towards ergosterol**.

Fig. 1

The iron regulon influences susceptibility to fluconazole

Aft1 is a transcriptional regulator which induces transcription of iron recovery genes, upon iron starvation. Overexpression of *MGE1* does not reduce the susceptibility to fluconazole in an *aft1* Δ strain. Fra1 is a negative regulator of the iron regulon. If **activation of the iron regulon** was the sole mechanism by which *MGE1* overexpression leads to fluconazole resistance, the *fra1* Δ strain should also show an increased MIC_{flu} compared to the wild type BY4742 strain. However, we show that this is not the case, indicating the **requirement of yet another, unknown, process** to work in conjunction with iron regulon activation in establishing fluconazole resistance downstream of Mge1.

Similar processes occur in *Candida* species

C. glabrata and *C. albicans* are two of the most frequently isolated pathogenic fungi in humans. Our results indicate that upon overexpression of *MGE1*, resistance of *C. albicans* (fig. 2) and *C. glabrata* to fluconazole is increased, similar to *S. cerevisiae*. In contrast to the latter organisms, however, the increased resistance in *C. albicans* seems to come at the cost of lower tolerance to the drug.

Fig. 2