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LETTER TO THE EDITOR

Trehalose as antifungal target: The picture is still incomplete

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A recently published review in *Virulence* by Perfect et al., surveys important topics regarding the use of the pathways involved in the metabolism of the non-reducing disaccharide trehalose, as a promising candidate in the search of new, safer and more potent antifungals. Much of the reported experimental data have mainly been taken from studies on *Crytptococcus sp*.[1] However, in my view, other crucial clues should be borne in mind for a better understanding of the reasons behind this proposal.

The critical control of the intermediate compound trehalose-6P (T6P) over glycolysis through the inhibition of hexokinase II initially reported in *S. cerevisiae* has been seen to be a common feature shared by many pathogenic fungi, including the opportunistic yeast *Candida albicans*,[2] which still remains the most prevalent fungal pathogen in humans. This significant finding opens alternative avenues for the development of new fungicidal strategies. The genetic evidence is also convincing, and the 2 essential genes involved in trehalose biosynthesis, namely T6P-synthase (*TPS1*) and T6P-phosphatase (*TPS2*) are themselves factors of virulence, while the corresponding *tps1*Δ and *tps2*Δ null mutants show a phenotype of hypersensitivity to severe oxidative stress, which is critical for *in vivo* tissue colonization.[3,4]

In fact, the involvement of trehalose as part of the fungal defensive response against antibiotics has already received consistent support. Thus, treatment with Amphotericin B induces a marked increase in trehalose synthesis in *C. albicans*, while the *tps1*Δ mutant is highly susceptible to the polyene.[5] A similar increase in trehalose was induced by miconazole and ciclopirox, but not by micafungin[6] (unpublished results).

More intensive research is required in order to lend weight to the proposal of incorporating trehalose into the antifungal chemotherapy arsenal, and conclusive in-depth clinical studies are particularly necessary. Another drawback is our still incomplete knowledge of the entire set of fungicidal mechanisms evolved by the families of chemotherapeutics currently used in medical practice. Interestingly, some compounds that interfere with trehalose metabolism have successfully been applied against phytopathogenic fungi. However, they failed to control infectious human yeasts. Furthermore, the regulatory roles played by tps1 protein in the central carbon metabolism, as well as the participation of trehalose in key physiological processes like the resumption of active growth from resting states and as defensive component against environment stress,[2,7] might complicate its putative fungicidal activity. In addition, the experimental evidence obtained from animal models is only suggestive and quantitatively insufficient.[1]

We need to focus on trehalose biosynthetic enzymes for future antifungal designs, since large amounts of intracellular trehalose are synthesized by many infective fungi (e.g. *C. albicans, Cryptococcus, Aspergillus* or *Magnaporthe*, among others) during *in vivo* tissue colonization.[1,2,7] In contrast, trehalose-hydrolyzing enzymes appear to play only minor roles in the fungal virulence composite, apart from a clear attenuation phenotype displayed by null mutants in mouse models.[1] It is worth noting, however, that the fungal cell wall confers a high degree of selective toxicity for new chemotherapeutic targets. In fact, clinical echinocandins act as potent inhibitors of β-glucan synthases. A close connection between trehalose metabolism and cell wall formation seems to be relevant in several pathogenic fungi. Thus, the so-termed acid trehalase (Atc1) must be considered a preferential antifungal target, since this enzyme is linked to the external cell wall in *C. albicans* and *C. parapsilosis*, and is involved in the hydrolysis of exogenous trehalose.[8,9] Furthermore, a *C. albicans tps1*Δ mutant shows high vulnerability to oxidative stress and diminished resistance to phagocytosis,

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but more importantly, it also displays serious defects in the cell-wall architecture.[10]

On the other hand, the potential use of trehalose metabolism as antifungal target mainly relies on the intriguing fact that the capacity to biosynthesize trehalose, strictly conserved throughout evolution in prokaryotes, lower eukaryotes, plants and invertebrates, has been absolutely abrogated in mammals.[7] This fascinating evolutionary hallmark makes the disaccharide an attractive antifungal target, but, at the same time, questions the strategy of using trehalase inhibitors applied inside the human body,[1] which contains 2 trehalase isozymes located as glycoproteins in the microvillus intestinal mucosa and renal brush-border membranes, respectively. This dual trehalase activity rapidly and completely degrades trehalose ingested in the diet, preventing its accumulation even in transitory or low levels.[7] Although far from conclusive, some evidence indicates that the disaccharide cannot be assimilated directly in the blood stream and its accumulation causes toxicity. Indeed, malabsorption due to altered intestinal trehalase gives rise to abdominal perturbations, diarrhea and other transitory digestive problems.[11] Additional analysis based on dietary trehalose would help clarify this matter.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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