

Unrealistic Nonphysiological Amounts of Reagents and a Disregard for Published Literature

Isaac Ginsburg

Institute of Dental Sciences, The Hebrew University, Hadash Faculty of Dental Medicine, Ein Kerem Campus, Jerusalem, Israel

Here are some comments and useful suggestions after reading an article in *mBio* by Brown et al. entitled “Mechanisms underlying the exquisite sensitivity of *Candida albicans* to combinatorial cationic and oxidative stress that enhances the potent fungicidal activity of phagocytes” (1).

In this paper, we are informed that a simultaneous exposure to 5 mM H₂O₂ and to cationic NaCl at 1 M is much more potent than the individual stresses themselves and that this combinatorial stress kills *C. albicans* synergistically *in vitro*. Such combinations are obviously absolutely unrealistic and not physiological. As a comparison I wonder why the authors had not also tested naturally occurring antimicrobial cationic peptides such as LL37 found in large amounts in neutrophil granules? Had the authors read the classical papers describing the possible mechanisms of bactericidal effects of neutrophils, they would have realized that there is actually no free-floating H₂O₂ in phagosomes following phagocytosis. This is because activation of NADPH-oxidase yields superoxide, which very rapidly interacts with myeloperoxidase (MPO) and with a halide (Cl⁻) to generate microbicidal amounts of hypochlorous acid (HOCl) (2–5)! Therefore, HOCl should have definitely been considered and tested in the system described by the authors. Also, the term flux used may be inappropriate since, in their study, both H₂O₂ and NaCl were actually applied as a bolus. Fluxes of oxidants are generated mainly by activated neutrophils and macrophages and by xanthine and xanthine oxidase in endothelial cells (2) Also, I wonder whether Na used is specific and whether potassium ions can also have the same effects in their system? The authors also claimed that catalase-derived peroxide detoxification, which is inhibited by cations, leads to intracellular ROS accumulation because catalase activity had been affected. If so, why had the catalase inhibitor azide or aminotriazole not been tested? In their study, the authors grew *Candida* cells in Tris-buffered yeast extract-peptone-dextrose medium (YPDT; pH 7.4). However, the authors have not cited key papers showing that D-glucose, in media on which candida grow, may also suppress catalase formation (6, 7).

Using unrealistic, *nonphysiological* amounts of reagents will not increase our understanding of how biological processes really occur *in vivo*, despite the need to employ *in vitro* models. Also,

disregarding key published data on neutrophil functions and *Candida* biology is unacceptable. Can this be a “menace to the future of honest science” (8) and also a “transgression” (9)? See also a recent publication by Casadevall and Fang (10).

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Address correspondence to Ginsburg@mail.huji.ac.il.