H2S AND NO AFFECT INTRACELLULAR KILLING OF *CANDIDA ALBICANS* MODULATING GSH LEVELS

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Candida albicans, a fungal microrganism, is involved in systemic infections in immunocompromised host inducing high martality. In macrophages, responsible for control of candida growth, redox status regulates the intracellular killing. Glutathione (GSH), the major intracellular antioxidant, and nitric oxide (NO) are important mediators in various physiological and pathological conditions including infections. Recent studies, also, show that endogenous hydrogen sulphide (H_2S) could protect cells from oxidative stress by modulating reactive oxygen species and scavenging peroxynitrite. In this study, the role of GSH on NO production during *Candida Albicans* infection and the relationship between the GSH, H_2S and NO in infected J774.1 macrophages was examined.

Methods: J774.1 cells, infected with *C. albicans*, were maintained for 1h at 37°C, in culture medium with or without GSH precursors (NAC, N-acetylcysteine) or with glutathione biosynthesis inhibitors (BSO, Buthionine-sulfoximine). In addition, in infected macrophages H2S was induced with Sodium hydrosulphide (NaHS) or inhibited whit DL-propargylglycine (PAG). GSH concentration was spectrophotometrically assayed in the infected macrophage lysates. To determine whether GSH and H_2S were, also, important for the intracellular killing of *C. albicans*, infected J774A.1 were lysed and the surnatant was plated on Sabouraud dextrose agar. The number of viable *C. albicans* was quantified with 10-fold dilutions spread. In infected macrophages, NO production was assessed by expression of inducible nitric oxide synthase (iNOS), detected by Real Time PCR.

Results: Treatment with BSO significantly reduced intracellular killing of C. albicans and GSH concentration (20% of the control). NAC addition to infected macrophages, increased GSH levels (170% of control) and induced a significant reduction in the viability of the intracellular *C. albicans* ($P< 0.001$). NaHS caused an increase of GSH levels (150% of control) and a decrease of number of viable yeast, while the inhibition of endogenous H_2S with PAG reduced GSH concentration and intracellular killing ($P< 0.05$). In the presence of BSO, iNOS was increased by 2-fold respect to unstimolated cells ($P< 0.001$), while NAC decreased iNOS synthesis ($P< 0.05$). Moreover, NaHS significantly inhibited NO production, while PAG caused iNOS expression ($P< 0.001$).

Conclusions: GSH may be involved in killing of *C. albicans* and in control of opportunistic fungal infections. The change of glutathione concentration during infection with *C. albicans* could regulate iNOS mRNA steady state levels in murine macrophages. We have also provided to evidence that H_2S could modulate intracellular killing by increasing GSH levels and inhibiting NO synthesis.