Relevance of *Trypanosoma cruzi* Fe-superoxide dismutases in macrophage and cardiomyocyte infections

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*Trypanosoma cruzi*, the causal agent of Chagas Disease, affects 7-8 million people in Latin America (WHO, 2013) not being yet an effective treatment against this disease. After invasion, macrophages are among the first line of defense. Parasites have to deal with large amounts of superoxide radical (O$_2$•-) produced during phagocytosis (by activation of NADPH-oxidase) and, in cytokine-activated macrophages (iNOS induction), with nitric oxide (•NO) and peroxynitrite. In this context the parasite antioxidant defenses are a key factor to escape the host’s oxidative assault and to establish the infection. In this work we study the relevance of the Fe-superoxide dismutases (Fe-SOD) contents of *T. cruzi* during macrophage and cardiomyocyte infections. 

*Trypanosoma cruzi* cytosolic Fe-SODB-overexpressing parasites were generated using the plasmid pRIBOTEX and expression evaluated by Western-blot and immunocytochemistry, with an increase in protein expression of 3-5 folds more than wild type (wt) parasites. Fe-SODB-overexpressing parasites were more resistant to O$_2$•- and •NO fluxes than wt (growth rates: wt 86%; FeSODB 95% at day 3 after exposure) and had a lower intra-parasite peroxynitrite production due to O$_2$•- detoxification (detection of the oxidation product of dihydroethidium and of fluorescein boronate by flow cytometry). In the context of macrophage infection, the anion O$_2$•- produced in the phagosome (pH ≈ 5-6) could be partially protonated leading to the neutral radical HO$_2$• (pKa = 4,88) that can easily diffuse to parasite cytosol generating O$_2$•-.

To investigate this route we exposed the parasites to fluxes of O$_2$•- (2-4 μM/min, 20 min) generated by the xanthine/xanthine oxidase system at acidic and basic pHs (6 and 8 respectively). In wt parasites, aconitase inhibition and 2-hidroxiethidium generation (O$_2$•-specific product of dihidroethidium oxidation) was higher at pH=6 than that observed at pH=8. In Fe-SODB overexpressing parasites aconitase inhibition and 2-hidroxiethidium generation were lower than wt clearly suggesting that at the pH of the phagosome O$_2$•- can be protonated to HO$_2$•and diffusing into the parasite. The ability of Fe-SODB overexpressers to detoxify O$_2$•- radicals was evaluated in macrophage infections (cell line J774A.1 and from murine bone marrow) either resting (O$_2$•-) or activated for the simultaneous generation of •NO and O$_2$•-, and thus peroxynitrite. Parasites overexpressing Fe-SODB were more resistant to macrophage killing (measured as the number of amastigotes/cell) than wt. This enhanced resistance was lost in NADPH-oxidase knockout macrophages (from gp91-/- mice) indicating the participation of O$_2$•- in the parasite control. Mitochondrial Fe-SODA overexpressing parasites present similar infection yields in resting macrophages when compared to wt but interestingly, Fe-SODA survival was significantly higher in cytokineinactivated macrophages. This may indicate the ability of macrophage-derived •NO to inhibit parasite cytochrome oxidase with the consequent generation of intra-mitochondrial O$_2$•-.

Finally, the infection yields in cardiomyocytes (cell line H9C2) were similar but in the presence of pro-inflammatory cytokines or •NO donors Fe-SODB-overexpressing parasites were more infective than wt. These results suggest that Fe-SODs could be acting as virulence factors for the infection hypothesis that needs to be confirmed in the experimental animal model of Chagas Disease.

Key words: *Trypanosoma cruzi*, Fe-superoxide dismutase, virulence