PEROXIREDOXIN OVEREXPRESSION AND RESPONSE TO NIFURTIMOX IN *Trypanosoma cruzi*

**Gabriela Specker**\(^1,2\), Damián Estrada\(^1,2\), Dolores Piñeyro\(^1,2,3\), Carlos Robello\(^1,2,3\), Rafael Radi\(^1,2\) and Lucía Piacenza\(^1,2\)

\(^1\)Department of Biochemistry and \(^2\)Center for Free Radical and Biomedical Research, Facultad de Medicina, Universidad de la República. \(^3\)Institut Pasteur de Montevideo

**Introduction**

*Trypanosoma cruzi*, the intracellular parasite responsible for Chagas Disease (CD), represents a public health problem in Latin America. Current drugs available, nifurtimox (NFX) and benznidazol have limited efficacy in the chronic stage of CD.

**Objectives**

Our research focuses on the effects of the overexpression of *T. cruzi* antioxidant enzymes in NFX-induced toxicity towards the epimastigote stage.

**Materials and methods**

Mitochondrial membrane potential and oxidants production were evaluated by flow cytometry. Peroxiredoxin activity and presence of high molecular weight (HMW) assemblies were assessed immunochemically. Chaperone-like activity was followed by the GFP-refolding assay.

**Results and Discussion**

*T. cruzi* overexpressing cytosolic and mitochondrial peroxiredoxin (CPX and MPX respectively) showed enhanced resistance to NFX-treatment (7µM). After 3 days, loss of mitochondrial membrane potential was observed in wild type parasites but not in MPX and CPX overexpressers. Generation of oxidative stress was previously observed at high NFX doses and thus, participation of reactive oxygen species (ROS) was evaluated. We measured the levels of H\(_2\)O\(_2\) and O\(_2^-\)) generation and the ratio of dimer/monomer peroxiredoxin (as a surrogate of peroxiredoxin activity) after NFX-treatment. No changes in these parameters were observed suggesting that, at least in our experimental conditions, NFX is not generating a detectable oxidative stress and thus peroxiredoxin-mediated protection may be due to mechanisms independent of ROS detoxification. It has been shown that HMW-assemblies of typical 2-Cys-peroxiredoxins function as molecular-like chaperones. We analysed the presence of MPX/CPX aggregates, finding these aggregates in the MPX/CPX overexpressers after NFX-treatment. Parasite HMW-protein extracts, from MPX/CPX overexpressers and NFX-treated wt showed enhanced chaperone-like activity. Moreover, enhanced expression of MPX/CPX was observed in wt parasites exposed to NFX.

**Conclusion**

Our results suggest that oligomerization of *T. cruzi* peroxiredoxins protects parasites from NFX-induced protein unfolding. Future studies are being designed to unravel the mechanism by which the gain of chaperone-like-activity protects parasites from NFX-toxicity and to establish peroxiredoxin overexpression as a possible mechanism of drug-resistance.