

ENZYMATIC ACTIVITY OF TRYPANOPTHIONE REDUCTASE IN PROMASTIGOTES AND AXENIC AMASTIGOTES OF *Leishmania (Viannia) braziliensis*

SARA MARIA XAVIER-CRUZ¹, MARCELLA DA SILVA FIGUEIREDO, LUZIA MONTEIRO DE CASTRO CÔRTES¹ BERNARDO ACÁCIO SANTINI PEREIRA² GEOVANE DIAS-LOPES³, CARLOS ROBERTO ALVES¹

¹FUNDAÇÃO OSWALDO CRUZ, RIO DE JANEIRO, BRAZIL, ²UNIVERSIDADE FEDERAL FLUMINENSE, RIO DE JANEIRO, BRAZIL, ³UNIVERSIDADE ESTADUAL DO RIO DE JANEIRO, RIO DE JANEIRO, BRAZIL.

American tegumentary leishmaniasis is a neglected disease caused by *Leishmania* parasites. The enzyme trypanothione reductase (TR) is essential for the redox balance of these organisms, making it a strategic target for innovative therapies and studies on antimonial resistance, a critical phenomenon that may negatively impact the current first choice treatment for such intentions. This study aimed to explore TR enzymatic activity in promastigotes and axenic amastigotes of 10 *L. (V.) braziliensis* isolates, classified as responders and non-responders to meglumine antimoniate treatment. The parasites were maintained *in vitro* as promastigotes and induced to undergo amastigogenesis by altering pH (7.2 to 5.5) and temperature (26°C to 32°C). Parasite samples were treated with lysis buffer for total protein extraction, and protein concentration was measured using the Qubit™ Protein Assay Kit. TR activity was assessed in the presence of NADPH, T[S]₂, and DTNB by spectrophotometry (412 nm, 35 min, 27°C). Results showed a time-dependent effect on TR activity stabilization. Kinetic curves reached stability between 14 and 23 minutes for promastigote proteins and between 16 and 25 minutes for axenic amastigote proteins, defining a 15-minute reaction interval for subsequent analyses. The lowest activity was observed in Isolate 6, which then was used as a reference sample for normalizing results. An additional analysis compared isolates as groups based on their treatment response phenotype. While isolates showed statistically significant differences when compared individually, no significant difference was found when comparing groups based on the resistance phenotype. These results suggest that variation in enzymatic activity may reflect differences in TR expression, with potential implications for the parasite's ability to cope with oxidative stress and adapt to the host environment.

Keywords: *Leishmania (Viannia) braziliensis*, trypanothione system, trypanothione reductase, enzymatic activity, antimony

Supported by: CNPq (301744/2019-0; 441634/2024-9), FAPERJ (E-26/010.000983/2019; E-26/200.799/2021; E-26/204.189/2021) e CAPES: 001.