INVOLVEMENT OF ADENOSINE AND GUANOSINE NUCLEOSIDES IN CELLULAR SIGNALING INVOLVED IN TRICHOMONIASIS PATHOGENESIS

Micheli Ferla; Tiana Tasca

Grupo de Pesquisa em Tricomonas, Laboratório de Pesquisa em Parasitologia, Faculdade de Farmácia, UFRGS, Porto Alegre, RS, Brasil.

INTRODUCTION

Trichomonas vaginalis is a flagellated protozoan that parasitizes the human urogenital tract, and causes the most common non-viral sexually transmitted infection (STI) in the world. Trichomoniasis is a public health problem due to its association with health complications, such as predisposition to cervical cancer and pelvic inflammatory disease in women. In men, the main complications are prostatitis and aggressive prostate cancer. Trichomoniasis is associated with both transmission and acquisition of HIV. The only two FDA-approved drugs for the treatment of trichomoniasis, metronidazole and tinidazole, belong to the same class, 5-nitroimidazole. However, metronidazole therapy has shown failure to eliminate infection, in this context, understanding the host-parasite relationship in order to investigate new agents is essential. The purinergic system is a cellular signaling network where nucleotides and nucleosides are regulated by enzymes called ectonucleotidases, and can bind to specific receptors called purinoceptors. The ectonucleotidases modulate the concentrations of extracellular nucleotides and nucleosides that regulate purine and pyrimidine receptors activation and consequently, the inflammatory responses. Studies on purinergic signaling are essential for the understanding of pathogenicity mechanisms and for the investigation of new therapeutic targets. The aim of this study was to evaluate the effect of serum restriction on nucleoside triphosphate diphosphohydrolase (NTDase) and ecto-5'-nucleotidase (E-5N) activities, simulating adenosine restriction.

MATERIALS AND METHODS

Adult bovine serum deprivation condition

Kinetic growth curve experiments were performed with the different clinical isolates in order to investigate the influence of heat inactivated bovine serum (HIBS) limitation on *T. vaginalis* growth, with an initial inoculum of 1.0 x 10^5 trophozoites/mL, in the presence of HIBS 1.0% (v/v) in TYM medium.

NTPDase and E-5N enzymatic assays

Specific activity was measured by determining the release of inorganic phosphate (Pi) by a colorimetric test. Trophozoites were washed three times with glycated saline (0.9-0.2%) and the Coomassie Blue method was used to determine protein quantification. The suspensions were diluted to have a final protein concentration of 0.6 mg/mL. To measure ATP and ADP hydrolysis, trophozoites were added to the reaction mixture containing 50 mM Tris pH 7.2 buffer and 5.0 mM $CaCl_2$ (ATP and ADP); to measure AMP hydrolysis, the reaction mixture was 50 mM Tris pH 7.5 buffer and 5.0 mM $MgCl_2$. The reaction initiated by the addition of ATP, ADP (1.0 mM) or AMP (3.0 mM) to determine NTPDase and E-5N activities, respectively. The reaction was stopped by the addition of 10% TCA. The experiment was done in triplicate three times.

LDH release assay

A timeline curve was performed to determine the maximum release of LDH over time using the Cyto Tox-One (Promega, USA). On the previous day, 3.0 x 10^4 cells were seeded in a 96-well microplate and maintained at 37°C and 5% CO_2 until monolayer confluency. Parasites in the logarithmic phase of growth exhibiting normal morphology and motility were washed three times and were resuspended on a new DMEM medium. An aliquot of 100 mL from a solution containing 5.0 x 10^5 trophozoites/mL was added to confluent DU145 cells and incubated during six hours at 37°C and 5% CO_2. The background was expressed as LDH release by DU145 unexposed cells. Data were expressed as a percentage of total lysis, using as control LDH release after 0.2% Triton x-100 exposed.

RESULTS

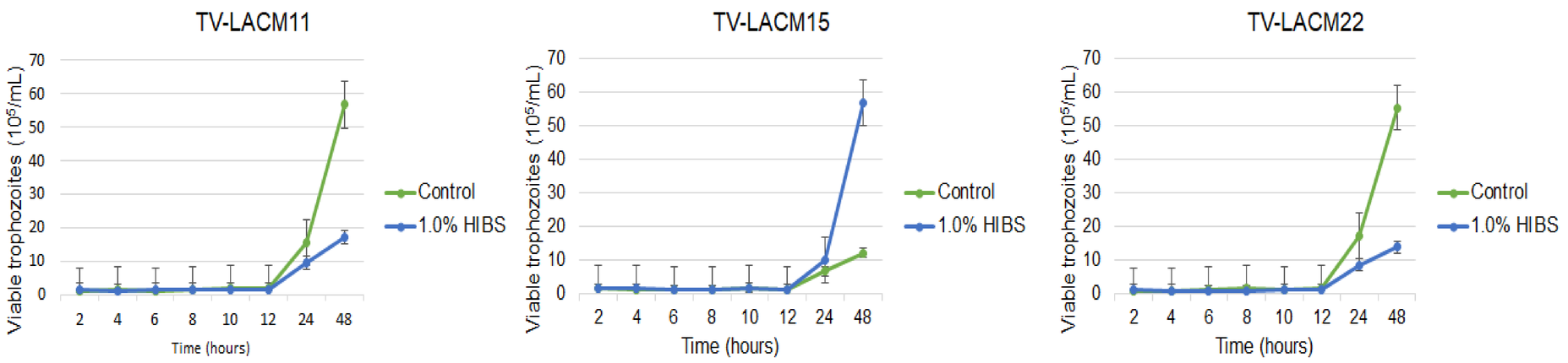

Fig. 1: Effect of 1% HIBS on *T. vaginalis* kinetic growth assay. All isolates showed decrease in growth in relation to control. Data represent media ± standard deviation of trophozoite counting in relation to control.

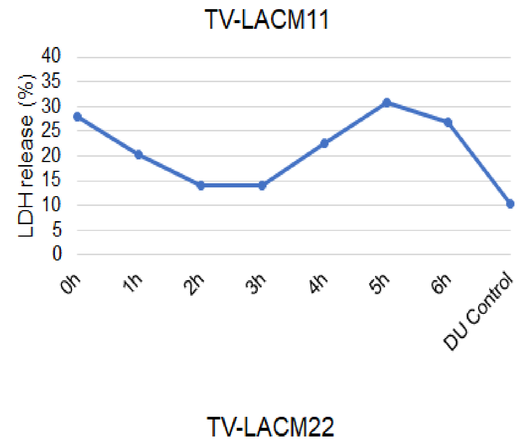

Fig. 3: LDH release assay. TV-LACM11 and TV-LACM22 showed a higher release of LDH in 5 hours.

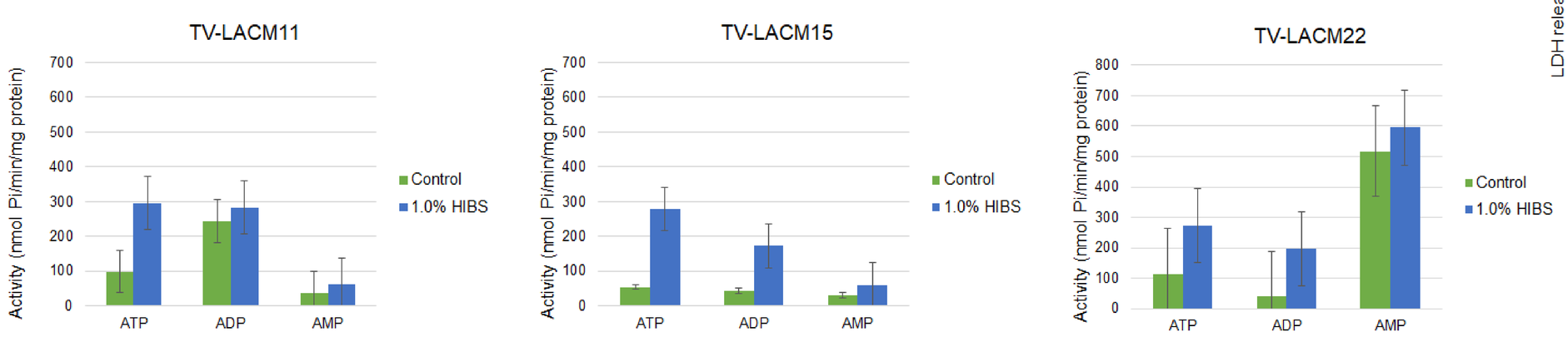

Fig. 2: Effect of 1.0% HIBS on NTPDase and E-5-N. Results show an increase in ATP, ADP, and AMP hydrolysis. Data represent media ± standard deviation.

CONCLUSIONS

The isolates showed decreased growth when maintained in 1.0% HIBS after 24h, time chosen for enzyme assays. The serum restriction increased NTPDase and E-5N activities in all *T. vaginalis* isolates. The highest LDH release in isolates occurred in 5h. The development of this study will contribute to the investigation of an important biochemical system for parasite survival that may be essential for the establishment of infection and thus may become a new therapeutic target.

REFERENCES

Menezes, Camila Braz, et al. *Microbes and infection* (2017) 19:122-131.
Menezes, Camila Braz, et al. *Molecular and biochemical parasitology* (2016) 207:10-18.
Rowley, Jane, et al. *Bulletin of the World Health Organization* (2019) 97:548.

Acknowledgements

CNPq CAPES