Effect of serum restriction on the enzymatic activity of Ectonucleotidases of *Trichomonas vaginalis*

Ferla, M.[1*]; Tasca, T.[2]
[1,2]Grupo de Pesquisa em Tricomonas, Faculdade de Farmácia/UFRGS, Porto Alegre/RS
***micheli.ferla@ufrgs.br;**

Introduction: *Trichomonas vaginalis* is a flagellated protozoan that causes trichomoniasis, the most common non-viral sexually transmitted infection in the world. Trichomoniasis is considered a public health problem and is related to the transmission and acquisition of HIV. There are only two FDA-approved drugs to treat it: metronidazole and tinidazole. Understanding the host-parasite relationship, as well as purinergic signaling, is essential for understanding pathogenicity mechanisms and investigating new therapeutic targets. The aim of this study was to evaluate the effect of heat inactivated bovine serum (HIBS) restriction in *T. vaginalis* on the activities of nucleoside triphosphate diphosphohydrolase (NTDase) and ecto-5'-nucleotidase (E-5N), simulating adenosine restriction. Methods and results: Culture *in vitro*: The clinical isolates of *T. vaginalis*, TV-LACM11, TV-LACM15, and TV-LACM22 were cultured *in vitro* in trypticase-yeast extract-maltose (TYM) medium, pH 6.0, supplemented with 10% (v/v) HIBS, and incubated at 37°C. HIBS deprivation condition: Kinetic growth curve experiments were performed with the different clinical isolates in order to investigate the influence of HIBS limitation on *T. vaginalis* growth, with an initial inoculum of $1x10^5$ trophozoites/mL, in the presence of HIBS 1.0% (v/v) in TYM medium. All 1.0% HIBS-treated isolates showed lower numbers of trophozoites in relation to control up to 48h. 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 with saline-glucose (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 $CaCl_2$; to measure AMP hydrolysis, the reaction mixture was 50 mM Tris pH 7.5 buffer and 5.0 mM $MgCl_2$. The reaction was initiated by the addition of ATP, ADP (1.0 mM) or AMP (3.0 mM) to determine NTPDase and E-5N activities, respectively, and was stopped by adding 10% TCA. The NTPDase activity more than tripled in 1.0% HIBS-treated parasites, while the E-5N activity increased slightly. Assays to determine the effect of HIBS limitation in adenosine deaminase activities, and the cytotoxicity exerted by *T. vaginalis* against host cells are in progress. Conclusion: HIBS restriction led to decreased parasite growth, while NTPDase and E-5N had an activity increase. This suggests that the purinergic system could be important in the establishment of infection and could thus be a therapeutic target.

Keywords: *Trichomonas vaginalis*; Purinergic signaling; Serum restriction

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