Involvement of adenosine in serum restriction in cellular signaling involved in trichomoniasis pathogenesis

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Introduction: *Trichomonas vaginalis* is a protozoan that causes trichomoniasis, the most common non-viral STI in the world. Trichomoniasis is related to the transmission and acquisition of HIV and is a public health problem. There are only two FDA-approved drugs to treat it: metronidazole and tinidazole. To understand the host-parasite relationship, as well as purinergic signaling, is essential the study of pathogenicity mechanisms to search for new therapeutic targets.

Objectives: 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 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 caused decrease in 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 potential therapeutic target.

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