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

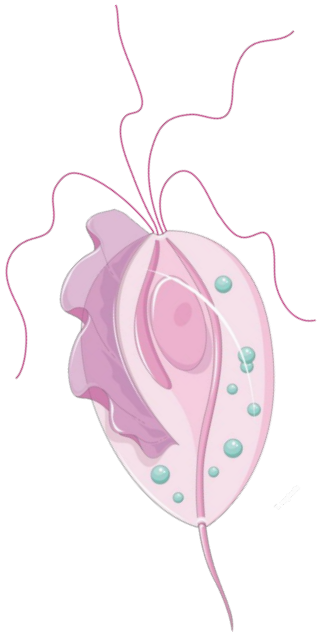
Micheli Ferla, Tiana Tasca

Grupo de Pesquisa em Tricomonas
Faculdade de Farmácia
Universidade Federal do Rio Grande do Sul

INTRODUCTION

Trichomonas vaginalis

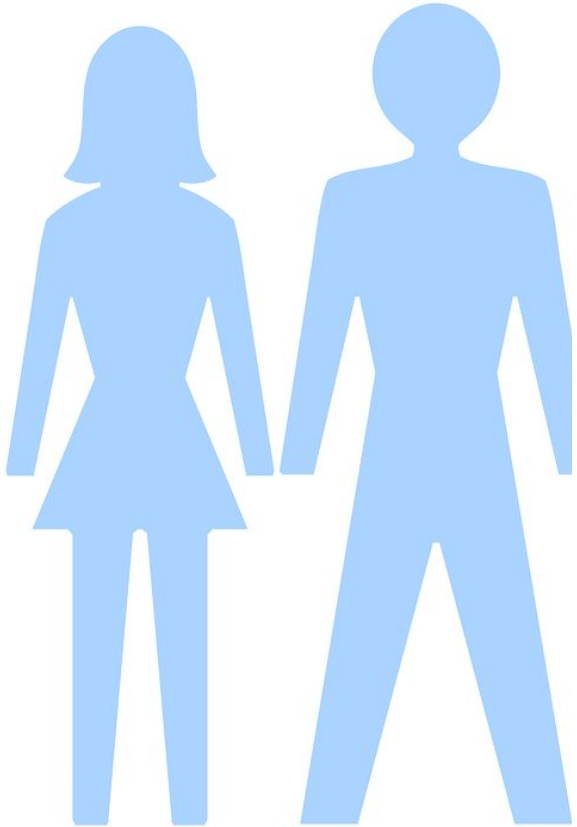
- flagellated protozoan
- agent of trichomoniasis
- the most common non-viral sexually transmitted infection (STI) in the world
- neglected parasitic infection
- increase of HIV acquisition and transmission
- prevalence of 110.4 million cases



(Edwards et al., 2016; Kissinger et al., 2013; Rowley et al., 2019)

INTRODUCTION

80% of cases are asymptomatic



WOMEN

pruritus
vaginal discharge
colpitis macularis or strawberry cervix

Complications such as:

- preterm delivery
- low birth weight
- pelvic inflammatory disease
- infertility
- cervical cancer

MEN

urethritis

Complications such as:

- infertility
- prostate cancer

(Petrin et al., 1998; Poole et al., 2013)

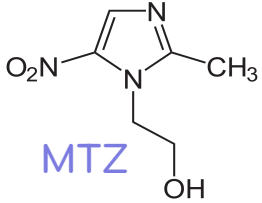
INTRODUCTION

Treatment of trichomoniasis:

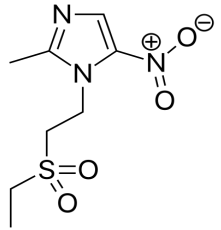
- the only 2 drugs recommended by the Food and Drug Administration (FDA, USA)

➔ metronidazole (MTZ)
➔ tinidazole (TNZ)

- both drugs belong to the 5-nitroimidazole class
- therapeutic failures

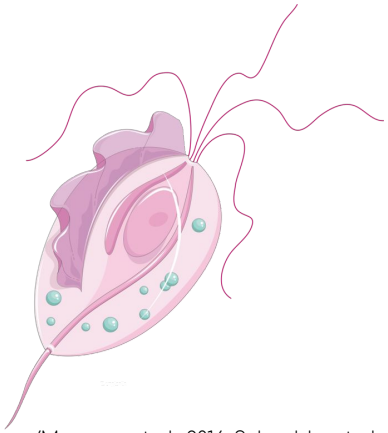


MTZ



TNZ

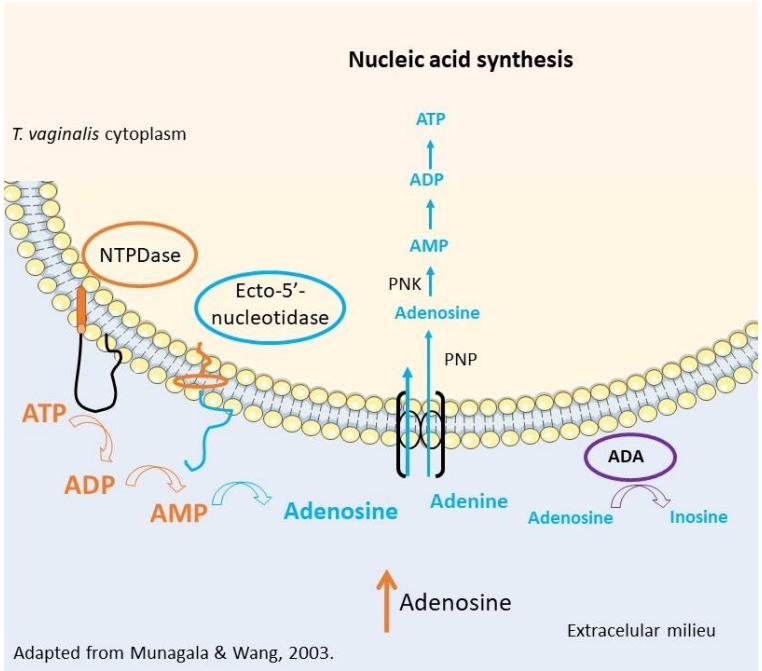
RESISTANCE OF 5-NITROIMIDAZOLE DRUGS ESTIMATED AT 2.5% - 9.6%



(Menezes et al., 2016; Schwebke et al., 2006)

INTRODUCTION

- purinergic system is a cellular signaling network
- nucleotides and nucleosides are regulated by enzymes called ectonucleotidases
- NTPDase catalyzes the degradation of nucleotides tri- and diphosphate and E-5N hydrolyzes monophosphate nucleosides
- bind to specific receptors called purinoceptors, whose activation alters cellular immune function



(Burnstock, 2014; Munagala & Wang, 2003)

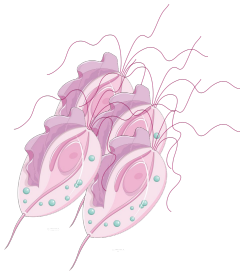
GOAL

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

HIBS deprivation condition:

TVLACM11
TVLACM15
TVLACM22



initial inoculum of $1x10^5$ trophozoites/mL, in the presence of HIBS 1.0% (v/v) in TYM medium

incubated at 37°C, pH 6.0

1:1 trypan blue

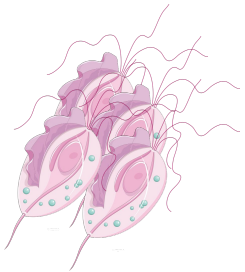
measured after: 2, 4, 6, 8, 10, 12, 24 and 48 hours

★ The same inoculum was prepared in parallel to the control group (10% v/v serum).

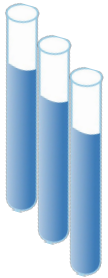
METHODS

NTPDase and E-5N enzymatic assays:

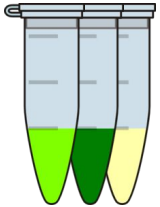
TVLACM11
TVLACM15
TVLACM22



Trophozoites were washed with glucose-saline (0.9-0.2%)



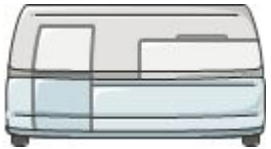
Coomassie Blue method was used to determine protein quantification (final protein concentration of 0.6 mg/mL)



To measure ATP and ADP hydrolysis (50 mM Tris pH 7.2 buffer and 5.0 mM CaCl2); ATP, ADP (1.0 mM) to determine NTPDase

To measure AMP hydrolysis (50 mM Tris pH 7.5 buffer and 5.0 mM MgCl2); AMP (3.0 mM) to determine E-5N activities

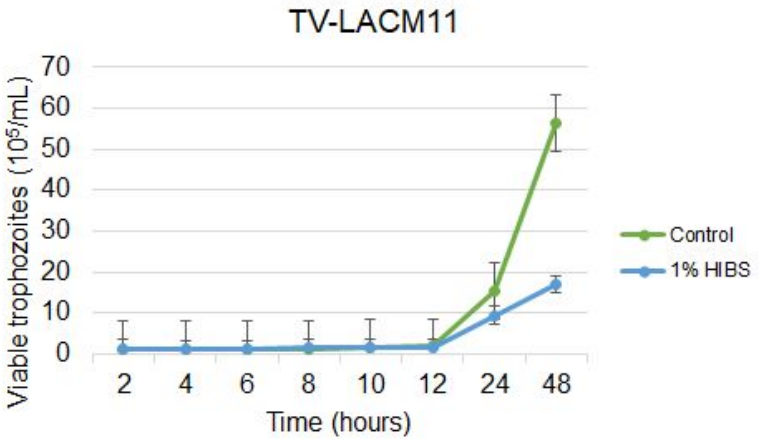
The reaction was stopped by adding 10% TCA

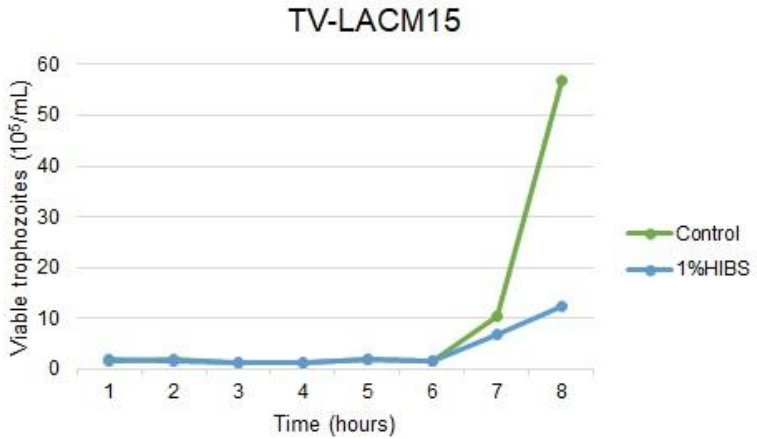


The NTPDase and E-5N activities were determined

RESULTS

HIBS deprivation condition:





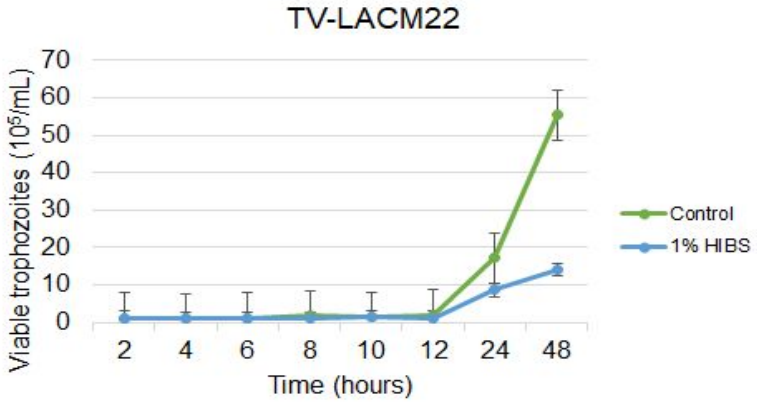
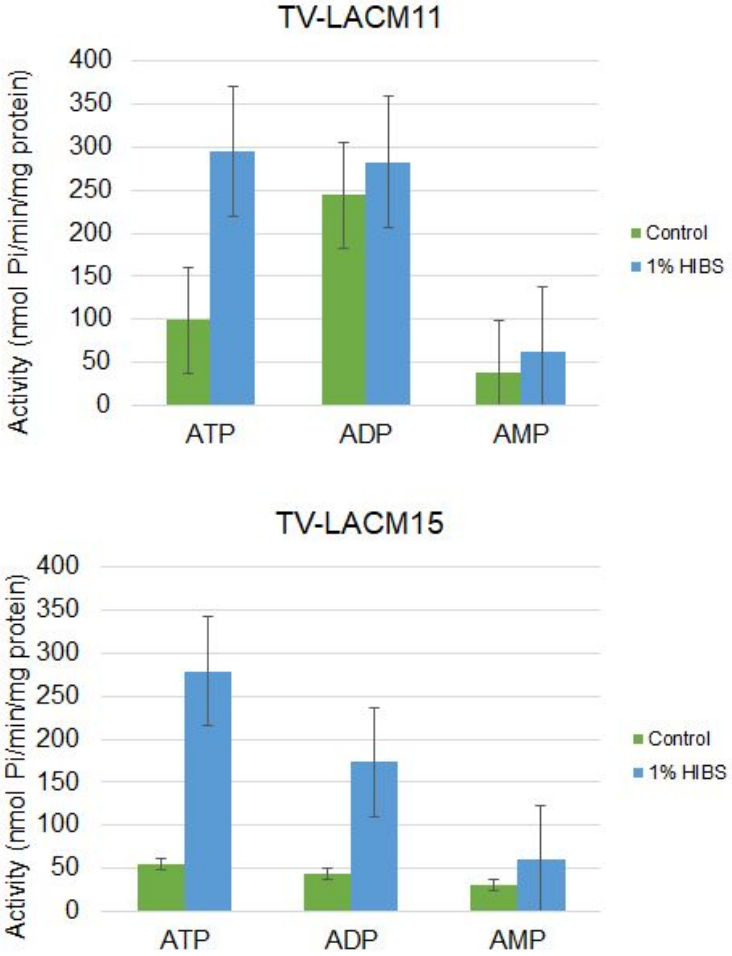


Fig. 1: Effect of 1% HIBS on *T. vaginalis* kinetic growth assay. All 1.0% HIBS-treated isolates showed lower numbers of trophozoites in relation to control up to 48 h.

RESULTS

NTPDase and E-5N enzymatic assays:



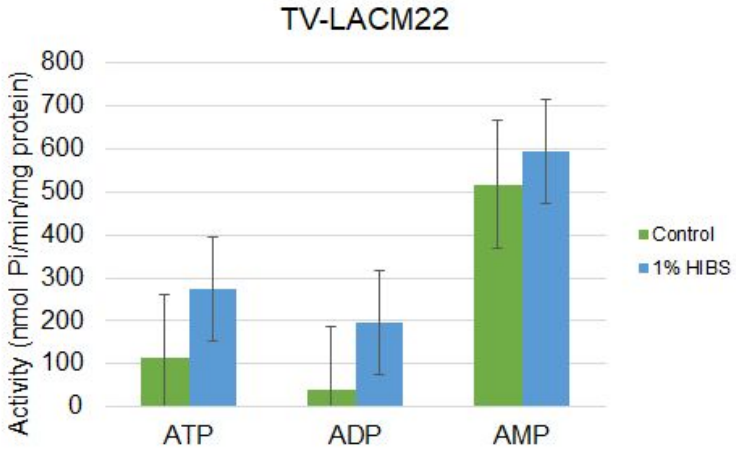


Fig. 2: Effect of 1.0% HIBS on NTPDase and E-5N. Results show an increase in ATP, ADP, and AMP hydrolysis. Data represent media ± standard deviation.

CONCLUSIONS

- ★ HIBS restriction led to decreased parasite growth
- ★ 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

ACKNOWLEDGEMENTS