



CLUBE DE PURINAS
BRASIL

X MEETING OF THE BRAZILIAN PURINE CLUB



INVOLVEMENT OF ADENOSINE IN SERUM RESTRICTION IN CELLULAR SIGNALING INVOLVED IN TRICHOMONIASIS PATHOGENESIS

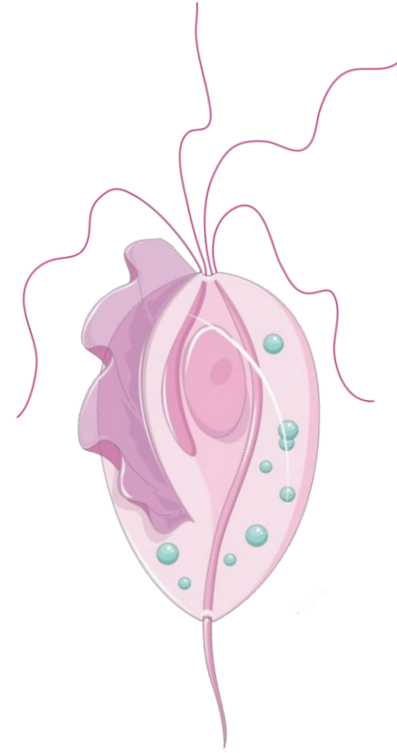
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INTRODUCTION

Trichomonas vaginalis

- Flagellated protozoan
- Parasitizes the human urogenital tract
- Agent of trichomoniasis
- The most common non-viral sexually transmitted infection (STI) in the world
- Neglected parasitic infection
- Increase in HIV transmission and acquisition
- Prevalence of 110.4 million cases



INTRODUCTION

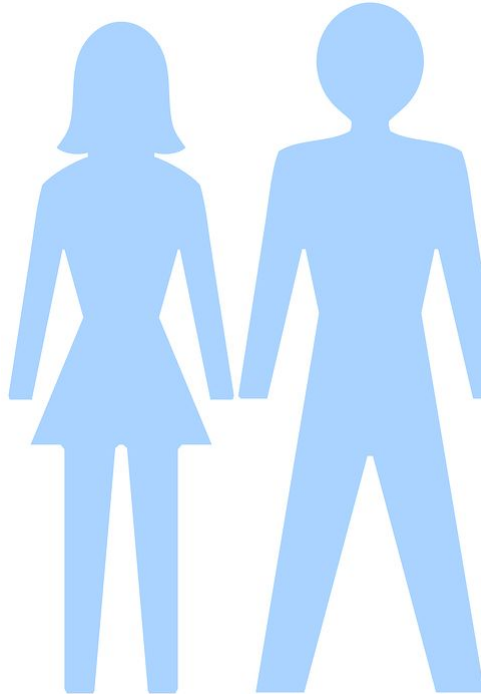
80% of cases are asymptomatic

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

INTRODUCTION

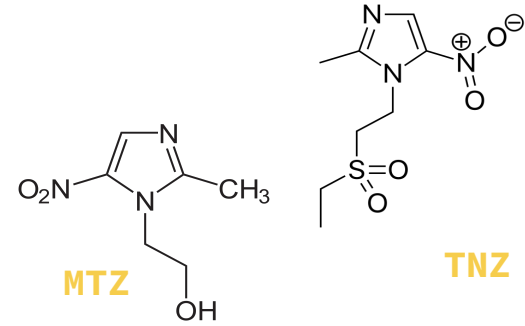
✦ Treatment of trichomoniasis:

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

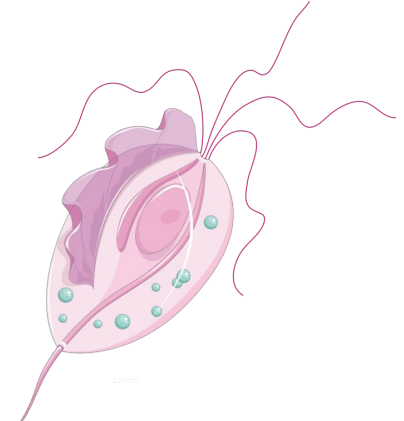
metronidazole (MTZ)

tinidazole (TNZ)

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



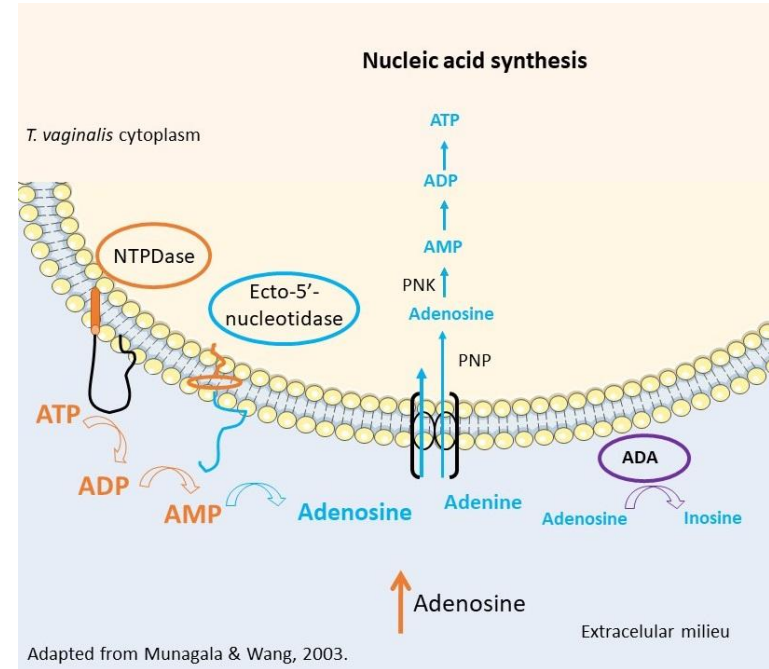
RESISTANCE OF
5-NITROIMIDAZOLE DRUGS
ESTIMATED AT 2.5% -
9.6%



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

INTRODUCTION

- The mechanisms involved in *T. vaginalis* pathogenicity and immune evasion are complex
- Besides NTPDase and E-5N activities, the presence of adenosine deaminase (ADA) in *T. vaginalis* to metabolize the product adenosine to inosine, presents anti-inflammatory effects
- Studies involving purinergic signaling in *T. vaginalis* help to understand the parasite biochemistry and host-parasite relationships



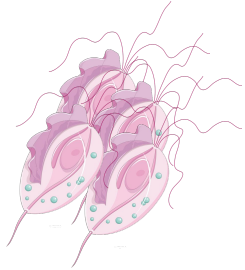
OBJETIVE

- ★ To evaluate the effect of heat inactivated bovine serum (HIBS) restriction, simulating adenosine restriction, in *T. vaginalis* NTPDase and E-5N activities.

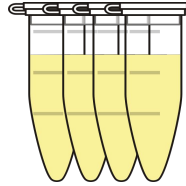
MATERIAL AND METHODS

HIBS deprivation condition:

TVLACM11
TVLACM15
TVLACM22



Initial inoculum of
 1×10^5 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

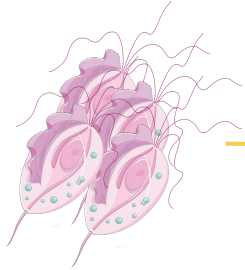


The same inoculum was prepared in parallel to the control group (10% v/v serum).

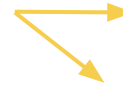
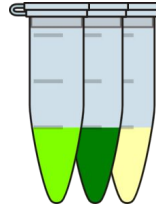
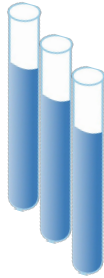
MATERIAL AND METHODS

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 CaCl_2); ATP,
ADP (1.0 mM) to determine
NTPDase

To measure AMP hydrolysis (50
mM Tris pH 7.5 buffer and 5.0
mM MgCl_2); AMP (3.0 mM) to
determine E-5N activities



The reaction
was stopped by
adding 10% TCA



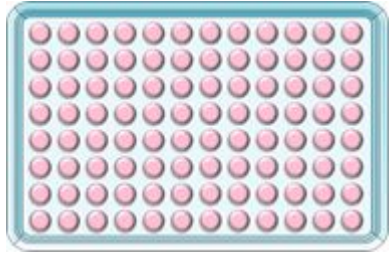
The NTPDase and
E-5N activities
were determined



MATERIAL AND METHODS

LDH release assay:

DU145



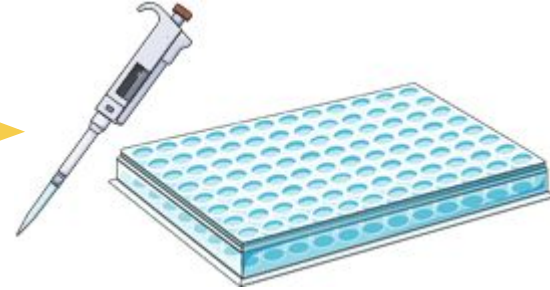
3.0×10^4 cells were seeded in a 96-well microplate and maintained at 37°C and 5% CO₂ until monolayer confluency

Data were expressed as a percentage of total lysis, using as control LDH release after 0.2% Triton X-100 exposed

*The background was expressed as LDH release by DU145 unexposed cells

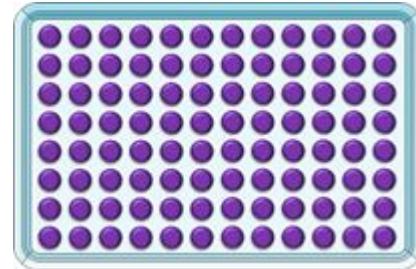


Parasites were washed three times and were resuspended on a new DMEM medium



An aliquot of 100 μ L from a solution containing 5.0×10^5 trophozoites/mL was added to confluent DU145 cells and incubated during 6h at 37°C and 5% CO₂

A timeline curve was performed to determine the maximum release of LDH over time using the CytoTox-One (Promega, USA)



RESULTS AND DISCUSSION

HIBS deprivation condition:

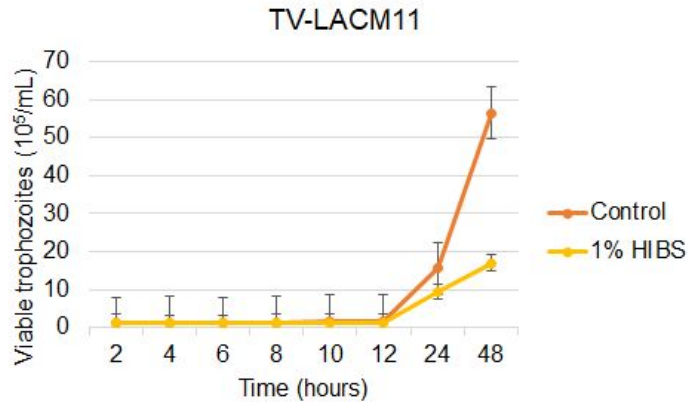
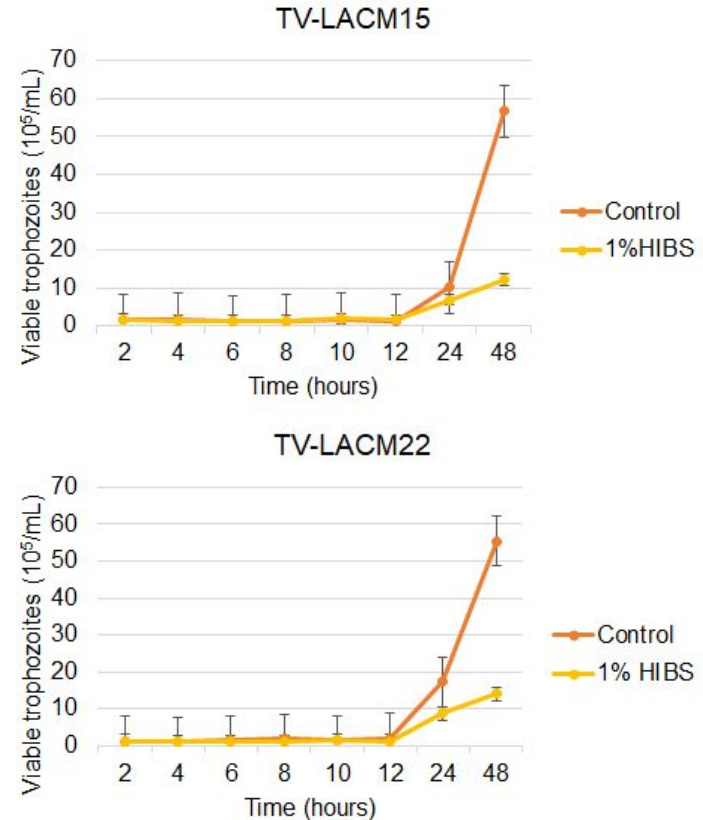


Fig. 1. Effect of 1% HIBS on *T. vaginalis* kinetic growth assay. All 1.0% HIBS-treated isolates showed lower numbers of trophozoites in relation to control up to 48 h.



RESULTS AND DISCUSSION

NTPDase and E-5N enzymatic assays:

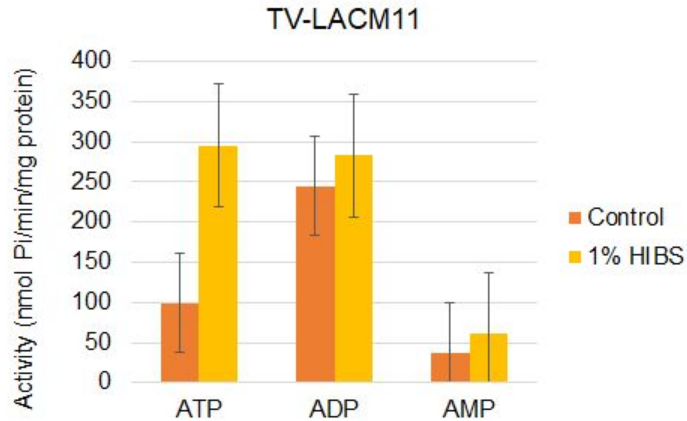
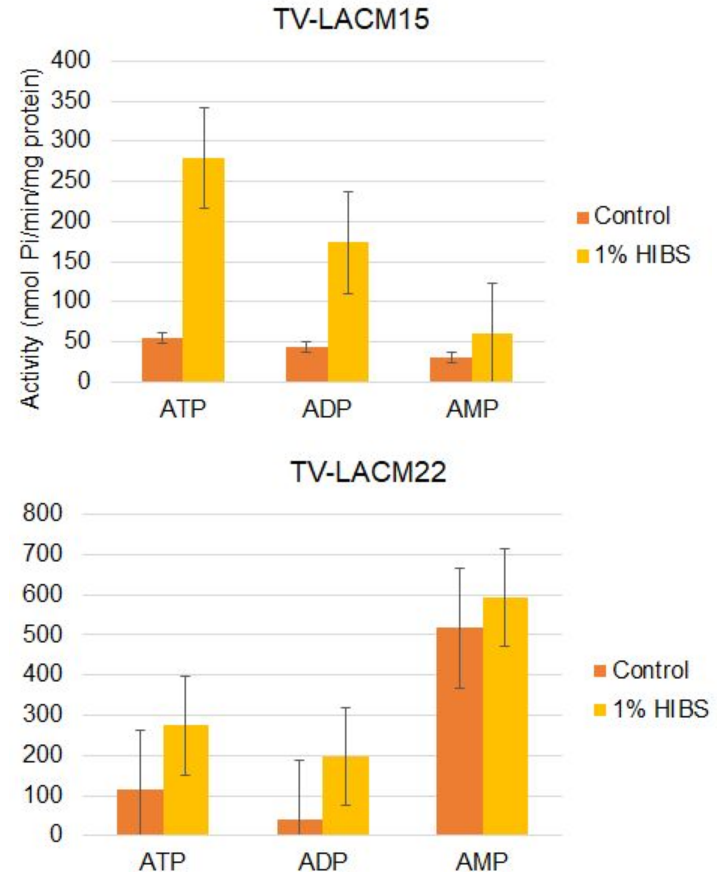


Fig. 2. Effect of 1.0% HIBS on NTPDase and E-5N. Results show an increase in ATP, ADP, and AMP hydrolysis. Data represent media \pm standard deviation.



RESULTS AND DISCUSSION

LDH release assay:

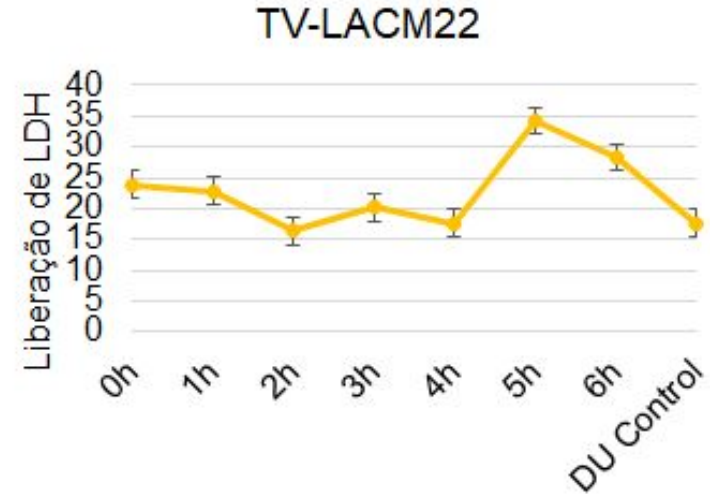
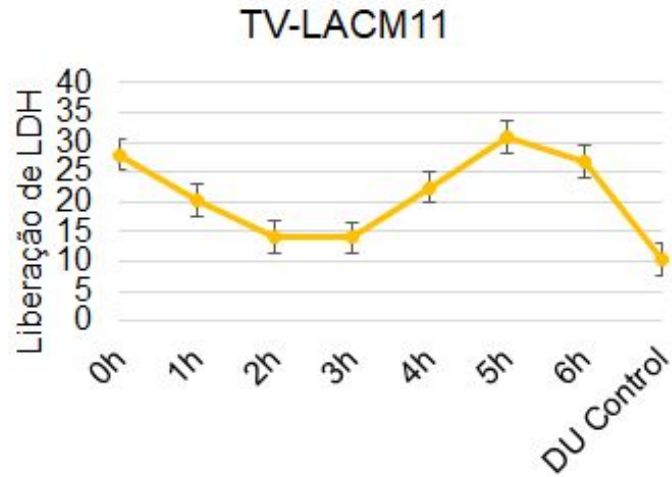


Fig. 3. LDH release assay in DU145 cells. TV-LACM11 and TV-LACM22 showed a higher release of LDH in 5 hours.

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

