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Original article

Adenosine, but not guanosine, protects vaginal epithelial cells from *Trichomonas vaginalis* cytotoxicity

Camila Braz Menezes [a,1], Amanda Piccoli Frasson [a,b,1], Lucia Collares Meirelles [a], Tiana Tasca [a,*]

[a] *Laboratório de Pesquisa em Parasitologia, Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul, Av. Ipiranga 2752, 90610-000, Porto Alegre, RS, Brazil*

[b] *Domo Salute Consultoria Regulatória Ltda, Rua Cristóvão Colombo 2948/411, CEP 90560-002, Porto Alegre, RS, Brazil*

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Abstract

Trichomonas vaginalis causes the most common non-viral sexually transmitted disease worldwide. The cytoadherence and cytotoxicity upon the vaginal epithelial cells are crucial for the infection. Extracellular nucleotides are released during cell damage and, along with their nucleosides, can activate purinoceptors. The opposing effects of nucleotides versus nucleosides are regulated by ectonucleotidases. Herein we evaluated the hemolysis and cytolysis induced by *T. vaginalis*, as well as the extracellular nucleotide hydrolysis along with the effects mediated by nucleotides and nucleosides on cytotoxicity. In addition, the gene expression of purinoceptors in host cells was determined. The hemolysis and cytolysis exerted by all *T. vaginalis* isolates presented positive Pearson correlation. All *T. vaginalis* isolates were able to hydrolyze nucleotides, showing higher NTPDase than ecto-5′-nucleotidase activity. The most cytotoxic isolate, TV-LACM6, hydrolyzes ATP, GTP with more efficiency than AMP and GMP. The vaginal epithelial cell line (HMVII) expressed the genes for all subtypes of P1, P2X and P2Y receptors. Finally, when nucleotides and nucleosides were tested, the cytotoxic effect elicited by TV-LACM6 was increased with nucleotides. In contrast, the cytotoxicity was reversed by adenosine in presence of EHNA, but not by guanosine, contributing to the understanding of the purinergic signaling role on *T. vaginalis* cytotoxicity.

Keywords: Trichomonas vaginalis; Cytopathogenesis; NTPDase; ecto-5′nucleotidase; Adenosine; Guanosine

1. Introduction

Trichomonas vaginalis is an obligate extracellular mucosal parasite and agent of the most common non-viral sexually transmitted disease (STD) worldwide with 276.4 million new infections a year [1]. Recent data have mentioned that around 80% of *T. vaginalis* infections are asymptomatic in both men and women [2]. The main health issues involving trichomoniasis are associated with adverse pregnancy outcomes, HIV acquisition, and cervical and prostate cancers [2–5]. The establishment of *T. vaginalis* infection is closely related to its ability to adhere to the host cells – epithelial cells from the vagina, cervix, urethra or prostate [6]. At the infection site, after the first contact with human cells, trophozoites undergo a drastic morphological shift, changing from the usual pear shape to an ameboid form [7,8]. Cytoadherence is mainly mediated by *T. vaginalis* lipophosphoglycan and five surface proteins characterized as adhesins (AP120, AP65, AP51, AP33, and AP23), as well as BspA-like proteins and other three membrane proteins characterized as tetraspanins [9–13].

In a second step, after cytoadherence to host cells the trophozoites start the mechanism of cytotoxicity resulting in cytolysis, phagocytosis, and disintegration of cell monolayers [8]. *T. vaginalis* displays an efficient ability to promote cytolysis followed by phagocytosis that culminate in disruption of cell monolayers [14]. The factors involved in the cytotoxic process

* Corresponding author. Fax: +55 51 3308 5437.
E-mail address: tiana.tasca@ufrgs.br (T. Tasca).
[1] These authors contributed equally to this work.

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include contact-independent mechanisms such as the *T. vaginalis* factor (TvF) that causes cell rounding and clumping without lysis and a glycoprotein with 200 kDa, known as cell-detaching factor (CDF) which promotes cell detachment [15]. In addition, high levels of proteolytic activity are attributed to secreted cysteine proteases (CP) [16]. *T. vaginalis* also effectively promotes hemolysis. Erythrocyte lysis constitutes an essential source of nutrients such as lipids and iron and may occur *in vivo* [17]. Surface cysteine proteases, pore-forming proteins, and phospholipase-A-like proteins also participate in the contact-dependent process [16–18].

Once triggered by *T. vaginalis* adhesion, host cells respond to the injury by the release of endogenous molecules and factors that will modulate and control the inflammatory process [19]. These molecules comprise extracellular ATP and the related purine and pyrimidine nucleotides that are passively released following cellular stress or cell death and exert their functions via signaling through membrane-bound purinergic P2 receptors [20]. On the other hand, nucleosides as adenosine often suppresses inflammatory cell responses, particularly adenosine A2A receptor (ADORA2A) has been reported to be a part of a negative feedback mechanism that limits local and systemic inflammation [21]. In the extracellular space, the nucleotide and nucleoside levels may be dynamically controlled by a group of membrane-bound enzymes named ectonucleotidases. These enzymes include the ectonucleoside triphosphate diphosphohydrolase (E-NTPDase) family, which hydrolyze nucleoside tri- and diphosphates, and the ecto-5′-nucleotidase, which hydrolyzes nucleoside monophosphates [22]. Adenosine deaminase (ADA) is another enzyme involved on purinergic cascade which is responsible for the conversion of adenosine to inosine [23]. In *T. vaginalis* trophozoites the activity of these enzymes upon adenine and guanine nucleotides was already demonstrated [24–27].

The role of ectonucleotidases in parasitic protozoa pathogenesis has been studied. Marques-da-Silva et al. [28] demonstrated that the conversion of ATP, a molecule with pro-inflammatory activity, into adenosine, which possesses immunomodulatory properties, may contribute to the establishment of infection by *Leishmania*. More recently, it has been shown that the Golgi-located NTPDase1 of *Leishmania major* is required for lipophosphoglycan elongation and normal lesion development [29]. In addition, Santos et al. [30] demonstrated the influence of ecto-NTPDase activity on *Trypanosoma cruzi* infectivity and virulence. Considering the study of ectonucleotidases function in *T. vaginalis* infection, some significant contributions have been made by our group. Trophozoites under conditions of serum limitation present enhanced NTPDase and ecto-5′-nucleotidase activities, suggesting a role in providing nucleosides (adenosine and guanosine) required for *T. vaginalis* growth [27,31]. Moreover, we showed the involvement of the adenosinergic system in the nitric oxide production by human neutrophils stimulated by *T. vaginalis* via A_{2A} receptor activation [32]. Iron from hemoglobin and hemin, a well known *T. vaginalis* virulence factor, modulates NTPDase and ecto-5′-nucleotidase activities [33]. Conversely, very low iron levels by treating the organisms with iron chelators modulate *T. vaginalis* ADA activity and gene expression [34]. These studies indicate the participation of the purinergic signaling in *T. vaginalis* pathogenesis.

In this context, the aim of the present study was to investigate the participation of *T. vaginalis* NTPDase and ecto-5′-nucleotidase on the cytopathogenesis against human vaginal epithelial cells (HVECs). We evaluated the parasite cytotoxicity through hemolysis, cytolysis by LDH release, flow cytometry and confocal microscopy, as well as the extracellular nucleotide hydrolysis profiles of fresh and long-term-grown (ATCC) *T. vaginalis* isolates. Furthermore, the influence of extracellular nucleotides and nucleosides on the modulation of the host cell cytotoxicity mediated by *T. vaginalis* was assessed. Finally, the gene expression of purinoceptors from the HVECs, HMVII cell lineage, was determined.

2. Materials and methods

2.1. Parasites and vaginal epithelial cell culture

In this study, fresh clinical *T. vaginalis* isolates were used: TV-LACM6, TV-LACM11, TV-LACM15, TV-LACM22, TV-LACM24 (from female patients), TV-LACH4, and TV-LACH6 (from male patients) obtained from urine from the Laboratório de Análises Clínicas, Faculdade de Farmácia, UFRGS, Brazil (approved by UFRGS Ethical Committee, project No. 18923); and the isolate ATCC 30236 from the American Type Culture Collection. Considering that *T. vaginalis* may harbor the endosymbionts Trichomonasvirus (TVVs) and *Mycoplasma hominis*, the isolates were classified as demonstrated in Table 1 [35]. Parasites were cultivated in trypticase-yeast extract-maltose (TYM), pH 6.0 supplemented with 10% inactive bovine serum at 37 °C [36]. HVECs line (HMVII) was used to analyze the *T. vaginalis* cytotoxicity. Cells were cultivated in RPMI 1640 medium supplemented with 10% inactive fetal bovine serum and 100 μg/mL penicillin-streptomycin at 37 °C and 5% CO_2.

2.2. Hemolysis assay

Blood samples obtained from volunteer donors (approved by UFRGS Ethical Committee, project No. 1.202.565) were centrifuged (250×*g*, 5 min), had their plasma discarded, and

Table 1
Trichomonas vaginalis isolates infected with *Mycoplasma hominis* and *Trichomonas vaginalis* virus [35].

Isolate	*M. hominis*	TVV1	TVV2	TVV3	TVV4
ATCC 30236	+	+	−	+	−
TV-LACM6	+	+	−	−	−
TV-LACM11	+	−	+	+	+
TV-LACM14	+	−	−	−	−
TV-LACM15	−	−	−	−	−
TV-LACM22	+	−	−	−	−
TV-LACM24	+	+	+	+	−
TV-LACH4	−	+	+	+	+
TV-LACH6	+	+	−	+	−