ARTÍCULO ORIGINAL

Microtubule cytoskeleton distribution using fluorescent taxoid in Tetratrichomonas didelphidis

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ABSTRACT

Tetratrichomonas didelphidis is a flagellated protozoan found in the intestine of opossums. The specimens were stained by the Giemsa method and by FLUTAX-2, an active fluorescent derivative of Taxol which binds to the $\alpha\beta$ -tubulin polymerized of microtubules of cells. Giemsa stain revealed the morphological features of trichomonads such as four anterior flagella, undulating membrane, axostyle and posterior flagellum. An intense fluorescence was observed in living trophozoites of *T. didelphidis* and *Trichomonas vaginalis* (used as control), incubated with FLUTAX-2. An analysis of the composition of the cytoskeleton of *T. didelphidis* will contribute to understanding the cellular morphology of the parasites.

Key words: *Tetratrichomonas didelphidis*, microtubule cytoskeleton, fluorescent taxoid.

INTRODUCTION

Few trichomonad species have a proven pathogenic potential for mammals and birds. In general, the species inhabiting trichomonad primary sites, i. e., the large intestine, appear to be non pathogenic. The species with proven pathogenic potential have evolved to inhabit areas other than the large intestine of their hosts¹. *Tetratrichomonas didelphidis* is a flagellate protozoan found in the intestine, cecum and colon, of the opossum *Didelphis marsupialis*², *D. albiventris*³, and *Lutreolina crassicaudata*⁴. The taxonomy of trichomonads has had a complex history. This species was first described with

the name *Trichomonas didelphidis*⁵. Subsequently, others authors described the species again using the correct genus, *Tetratrichomonas*⁵.

Microtubules are essential for the maintenance of cell shape and organization; for cytoplasmic transport, motility and division in eukariotic cells. In addition, they are targets of antimetabolic drugs, including colchicines, vinblastine, and Taxol (paclitaxel)⁶. Taxol blocks the cell division and caused its death⁷⁻⁹, being widely used as a microtubule stabilizing agent. It is employed in the treatment of ovarian, breast, lung, head and neck, cervix carcinomas and lymphomas¹⁰. Recently, Taxol is used in experiments of transient invagination of flagella by *Tritrichomonas foetus*

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Figure 1. Chemical structure of FLUTAX-2.

to stabilizing microtubules¹¹ and in the study of resistance to arsenite in *Leshmania donovani*¹². Fluorescent Taxol derivatives can be extremely useful in the study of cellular microtubules, as well as the mechanisms of microtubule assembly and stabilization by Taxol^{13,14}. FLUTAX-2¹⁵ (Figure 1), an active fluorescent derivative of Taxol, binds to $\alpha\beta$ -tubulin dimer polymerized. It interacts specifically with the Taxol binding site, probably at the outer microtubule surface¹⁶, making them both directly and rapidly observable under fluorescence microscope. Cytoskeleton of Ptk2 epithelial cells, U937 leukemia cells, neuro2A neuroblastoma cells, and *Trypanosoma cruzi* was stained with FLUTAX¹⁴.

There are few reports regarding the biological aspects of *T. didelphidis*^{2,4,17,18}. Take into account that the cytoskeleton of *T. didelphidis* is completely unknown, in this study we present the analysis of microtubule distribution in living trophozoites of *T. didelphidis* using the fluorescent taxoid FLUTAX-2. This probe, 7-O-[N-(4'-fluoresceincarbonyl)-L-alanyl]Taxol (FLUTAX-2), was obtained by the reaction of 7-O-(L-alanyl) Taxol with the corresponding amine-reactive fluorescent dye, as described¹⁵. FLUTAX-2 had ultraviolet (UV)-visible absorbance and fluorescence emission spectra in accordance with the respective chromophores. Analysis by high-performance liquid chromatography (HPLC) FLUTAX-2 showed a purity (228 nm) of 91% (0.3% Taxol). The concentration of FLUTAX-2 was determined spectrophotometrically in 0.5% sodium dodecyl sulphate

(SDS), 50 mM sodium phosphate buffer, pH 7.0, employing the practical extinction coefficient $\epsilon_{458\text{nm}} = 23,100 \pm 600 \text{ M}^{-1} \text{ cm}^{-1}$, obtained by weighing and by SDS solubilization of the taxoid.

MATERIALS AND METHODS

The TDLC01 strain of *T. didelphidis* used in this study was isolated from the swabbed rectum of *L. crassicaudata* and cultivated in Diamond's¹⁹ modified trypticase-yeast extract-starch (TYS) medium, without maltose and with starch solution, pH 7.5 (5 mg/ml)¹⁷. The cells were grown *in vitro* at 28°C (± 0.5) in TYS medium supplemented with 10 % (v/v) heat inactivated bovine serum, penicillin (1000 IU/ml) and streptomycin sulphate (1 mg/ml). Certain samples were frozen and maintained at -196°C with 5% (v/v) of dimethyl sulfoxide (DMSO) as previously described²⁰.

One *Trichomonas vaginalis* strain was used in this study as control for FLUTAX-2 binding²¹. The 30238 strain was obtained from the American Type Culture Collection (ATCC). The strain was cultivated axenically *in vitro* at 37°C, in trypticase-yeast extract-maltose (TYM), Diamond medium¹⁹ without agar, pH 6.0, supplemented with 10% heat inactivated bovine serum, penicillin (1,000 IU/ml) and streptomycin sulphate (1 mg/ml). The trichomonads in the logarithmic phase of growth exhibited more than 95% mobility and normal morphology.

Trophozoites of *T. didelphidis* and *T. vaginalis* strains were counted in a hemocyto-

Figures 2-3. Trophozoites of *Tetratrichomonas didelphidis* in Giemsa-stained specimens. Bar = 10 μ m. **Figures. 4-6.** Specific visualization of cellular microtubules with FLUTAX-2 in living *Tetratrichomonas didelphidis* and *Trichomonas vaginalis*. **Figures 4-5** show *T. didelphidis* (1,000 x), while **Figure. 6** shows *T. vaginalis* (400 x). af indicates the anterior flagella; ax the axostyle; b the blepharoplast; c the centrosome; n the nucleus; pf the posterior flagellum, and um the undulating membrane.

meter to obtain 10^6 cells/ml. Intact cells were incubated with FLUTAX-2 (1 μ M, final concentration) at 37°C, during 40 min, and observed in a Zeiss fluorescence microscope.

RESULTS AND DISCUSSION

Figures 2 and 3 are from *T. didelphidis* TDLC01 strain, stained with Giemsa as previously described³, to show the protozoan structure by light microscopy. There were four anterior flagella unequal in size in the mature individuals, and a well-developed undulating membrane extended almost the entire length of the body (Figures 2, 3). The undulating membrane often exhibits several bold waves and the outer margin of the membrane consists of the accessory filament, which extends into a free posterior flagellum (Figures 2, 3). There was a spheroidal, ellipsoidal, or ovoid nucleus situated in the anterior portion of the body (Figures 2, 3), clearly discernible in most living and stained specimens. Anterior to the nucleus was a blepharoplast where

the flagella were inserted (Figures 2, 3). Posterior to the nucleus the capitulum continues as a slender, hyaline, somewhat attenuating axostylar trunk that courses near the anteroposterior axis of the organism, its terminal segment projecting approximately one-third the cell length beyond the posterior surface of the flagellate (Figure 2). Both trichomonad species displayed an intense fluorescence in the axostyle, centrosome and flagella of living cells, strongly suggesting the microtubule composition of these structures (Figures 4 - 6). Figures 4 and 5 show the trophozoites of *T. didelphidis* after incubation with FLUTAX-2. Structures such as centrosome, axostyle and the anterior flagella jointed, can be observed (Figure 4). Figure 6, 30238 strain, show the same structures as above: the centrosome, the axostylar position through the cell shape and, the anterior flagella, arranged in two groups, as described in some organisms²².

As shown by Lopes et al²³, using specific monoclonal antibodies, *T. vaginalis* presents α -tubulin at the flagella, basal bodies and the

axostyle. $\alpha\beta$ -tubulin is present at the axostyle and flagella too. Our data are in accordance with them²³, since FLUTAX-2 binds to ab-tubulin dimmers, for both trichomonad species. The axostyle contains acetylated tubulin²³, a characteristic isoform of stable microtubules²⁴, contrary to the findings of others who claim that this structure is not permanent²⁵⁻²⁷.

The direct observation of microtubules with FLUTAX-2 in living *T. didelphidis* and *T. vaginalis* trophozoites presents an advantage over indirect immunofluorescence and does not require microinjection of individual cells to introduce the probe. As shown by Evangelio et al.¹⁴, it allows diverse morphological applications, including for example, the taxonomy and cell cycle characterization of flagellates and ciliates. Moreover, the major advantage of this method is the absence of cell fixation, and consequent death, which can mask the true morphological characteristics of living cells.

Our results showed the binding of FLUTAX-2 in two trichomonad species. An analysis of the composition of the cytoskeleton of *T. didelphidis* will contribute to understanding the biochemical aspects involved in cellular morphology and behavior as well as the mechanisms related to host-parasite interactions.

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