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Short Communication

Pentamycin shows high efficacy against *Trichomonas vaginalis*

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ABSTRACT

Trichomonas vaginalis is the causative agent of the venereal disease trichomoniasis, which is the most frequent non-viral sexually transmitted disease worldwide. Since the 1960s, metronidazole has been the standard treatment, however an increasing number of cases with metronidazole-resistant strains is being reported. In this study, pentamycin, a polyene antibiotic, was tested for its in vitro efficacy against *T. vaginalis* using four strains with varying metronidazole susceptibility. It was shown that pentamycin is highly active against *T. vaginalis* and that the effect is prompt and independent of underlying metronidazole resistance. The effective concentrations (EC values) after 1 h of treatment were in the range 1.74–2.62 μg/mL (EC_{50}) and 4.91–6.51 μg/mL (EC_{90}). Total eradication of trichomonads (EC_{100}) was achieved in all strains by treatment with 15 μg/mL (22 μM) for 1 h or with ≥1 μg/mL (≥1.5 μM) for 24 h. Long-term cultivation (12 months) under permanent drug pressure did not induce stable resistance against pentamycin in any of the strains tested. Pentamycin has been approved for intravaginal use and is a promising candidate for the topical treatment of trichomoniasis.

1. Introduction

Trichomonas vaginalis is the causative agent of trichomoniasis, with 153 million cases of prevalent infection and an estimated 248 million new cases per year [1]. Untreated trichomoniasis in women can lead to fulminant vaginitis, temporary sterility, preterm delivery, co-infection with other pathogens (e.g. *Candida albicans*) and also to a chronic stage of the disease with permanent inflammation linked to the emergence of neoplasia and cancer [2]. Moreover, a role in the spread of human immunodeficiency virus (HIV) has been attributed to *T. vaginalis* [3]. For more than 50 years, the nitroimidazole metronidazole has been the standard drug for treatment of trichomoniasis. However, metronidazole can have toxic side effects [2,4] and, moreover, resistance to metronidazole has been increasing in the past years and is the main reason for treatment failure [2,5]. Alternative drugs are tinidazole, another nitroimidazole, with possible cross-resistance to metronidazole, and amphotericin B (AmB) [5].

Pentamycin, mainly produced by members of the genus *Streptomyces*, is a polyene and thus is structurally related to AmB. It consists of a macrocyclic ring of carbon atoms with a series of five conjugated carbon–carbon double bonds, closed by lactonisation [6]. Discovered and described in 1958 [7], pentamycin has been tested against various, mainly fungal, pathogens, e.g. *C. albicans* [8,9]. It has also been described as an enhancer of the anticancer agent bleomycin [10]. Pentamycin has already been approved for the topical treatment of bacterial and fungal vaginitis, administered as a vaginal tablet, and was shown to be very well tolerated [11].

The aim of the current study was to investigate the efficacy of pentamycin against *T. vaginalis* in vitro. A further aim was to monitor whether resistance to pentamycin can be induced in trichomonads by long-term exposure to the drug.

2. Materials and methods

Four strains of *T. vaginalis* with varying susceptibilities to metronidazole were used in this study: ATCC 30001 (highly

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susceptible); ATCC 30236 (normally susceptible); TV2 (normally susceptible); and ATCC 50138 (resistant). Strains were cultivated axenically at 37 °C in pre-warmed modified TYM (tryptose–yeast–maltose) medium as described previously [12], containing 10% heat-inactivated horse serum, 3% Diamond Vitamin Tween 80 Solution (40×) and 500 U/mL penicillin (all from Sigma-Aldrich, Vienna, Austria), in 42 mL culture flasks under microaerobic conditions. Medium was changed every 2–3 days by transferring 100 μL of the respective liquid culture into a new flask previously filled with fresh pre-warmed TYM medium. Pentamycin, used at a purification grade of 94% (Ch.-B. C-017084-PRS-03; provided by Lumavita AG, Basel, Switzerland) and metronidazole (purchased from Sigma-Aldrich) were dissolved in dimethyl sulfoxide (DMSO) (Sigma-Aldrich) to provide 1% stock solutions (10 mg/mL). Working solutions were prepared by diluting the respective stock solutions 1:10 in TYM medium.

Microtitre assays were performed in 24-well plates (Nunc™; Thermo Scientific, Braunschweig, Germany) with 3 mL volume per well. Before inoculation, cells were counted with a Fuchs–Rosenthal haemocytometer (VWR, Vienna, Austria) and were adjusted to 10^6 cells/mL per well. Filled plates were pre-warmed at 37 °C for ≥30 min. Test series were run with eight dilutions of pentamycin by diluting the working solution to final concentrations of 0.5, 1, 2, 3, 4, 5, 10 and 15 μg/mL. Metronidazole was included as a positive control at a concentration of 50 μg/mL. The respective highest concentration of DMSO and cells in TYM medium (blank sample) were used as negative controls. The efficacy of pentamycin was evaluated by Trypan blue (Sigma-Aldrich) staining after 1, 3 and 6 h of incubation, whereby each strain was counted in parallel triplicates. Effective concentrations (EC_{50} and EC_{90} values) were calculated using SPSS v.12 (SPSS Inc., Chicago, IL) with linear regression/probit analysis. Results are expressed as the arithmetic mean and standard deviation.

3. Results and discussion

3.1. Cytotoxic effect of pentamycin against Trichomonas vaginalis

The effect of pentamycin on *T. vaginalis* is prompt, comparable with the effect of the antiseptic *N*-chlorotaurine [12]. As soon as 10 min after administration of pentamycin, the morphology and physiology of *T. vaginalis* cells changed dramatically. Treated cells appeared rounded and enlarged and showed blebbing and attenuated motility. With prolonged incubation time, cells finally lysed. This effect correlated positively with the applied concentration of pentamycin and negatively with cell density. Treatment with 15 μg/mL resulted in complete lysis of the parasites within 1 h (Fig. 1A and B).

Pentamycin is known to interact with membrane sterols. Other polyenes such as nystatin and AmB have been shown to cause membrane distortion and consequently leakage of cytoplasmic material and cell death in fungi [13].

3.2. Effective concentrations

As pentamycin is administered topically, we aimed to simulate these conditions in vitro by exposing parasites to high amounts of the drug for a rather short period of time. Pentamycin was found to be highly effective against *T. vaginalis*; treatment with 15 μg/mL (22 μM) for 1 h resulted in 100% eradication of all strains in vitro, and in some strains 100% eradication within 1 h was even achieved

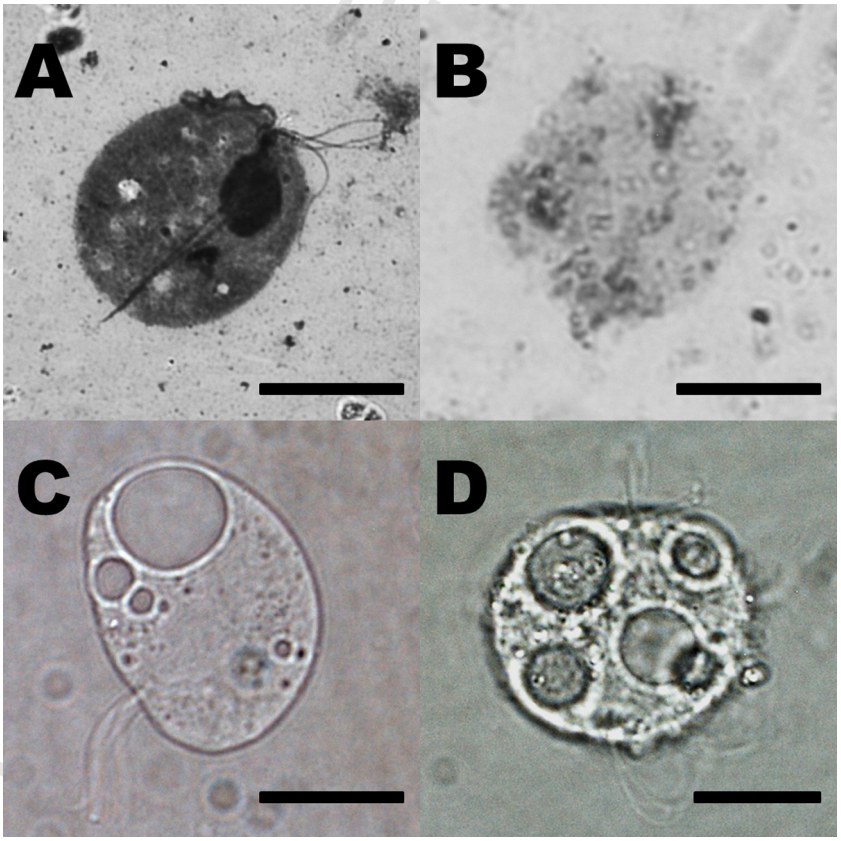

Fig. 1. *Trichomonas vaginalis* trophozoites: (A) untreated; (B) treated with 10 μg/mL pentamycin for 1 h; and (C and D) after long-term (12 month) treatment with sublethal concentrations. Bar = 10 μm.

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