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International Journal of Antimicrobial Agents 31 (2008) 282–286

INTERNATIONAL JOURNAL OF Antimicrobial Agents

www.ischemo.org

Short communication

In vitro effects of suramin on *Trypanosoma cruzi*

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Received 20 July 2007; accepted 6 November 2007

Abstract

Suramin has been previously reported to inhibit distinct cellular enzymes and to affect the synthesis and distribution of cytoskeleton proteins, cell differentiation and proliferation. The present study indicates that prolonged incubation of *Trypanosoma cruzi*-infected cells in the presence of 500 μM suramin during intracellular development of the parasite causes morphological changes in amastigote and trypomastigote forms related to the cell division and differentiation process. Our results also show that trypomastigotes obtained from suramin-treated host cells were significantly less infective than control parasites and that amastigotes derived from those trypomastigote forms were less proliferative.

Keywords: Suramin; *Trypanosoma cruzi*; Ultrastructure; Parasite–host cell interaction

1. Introduction

Trypanosoma cruzi is a parasitic protozoan belonging to the Trypanosomatidae family and is the aetiological agent of Chagas disease, a debilitating disease that is highly prevalent in Latin America with no immunoprophylactic agents available [1].

Standard chemotherapy regimens for Chagas disease possess limited efficacy during the acute and chronic phases and produce serious side effects. Therefore, new drugs have been developed based on the basic biology of *T. cruzi*. Among them are inhibitors of proteases and unique biochemical routes that have trypanocidal activity in vitro against intracellular amastigotes [2].

Suramin, a symmetrical polysulphonated derivative of urea, is an efficient drug in the prophylactic treatment of human trypanosomiasis in Africa. Suramin affects different proteins in different cell types, including the reverse transcriptase of retroviruses, protein kinase C, DNA polymerase, protein tyrosine phosphatases, lysosomal enzymes, ATPases and cytoskeleton components [3–6]. Furthermore, it acts as an antagonist of P_{2x} and P_{2y} purinoceptors and inhibits the binding of some growth factors to their receptors, which stimulated its use in cancer chemotherapy [7]. In trypanosomes, suramin inhibits several enzymes, endocytosis of some molecules and the binding of low-density lipoproteins to specific receptors [3,8], changes in the phenotypic expression of surface antigens and a redistribution of cell surface negative charges [3,5,9]. In the present study, we investigated under standardised conditions the in vitro activity of suramin against *T. cruzi*, taking into account the intracellular development and infectivity of trypomastigote forms.

2. Material and methods

2.1. Parasite

The Y strain of *T. cruzi* was used throughout this study. Tissue culture amastigotes and trypomastigotes were obtained from the supernatant of LLC-MK_2 cells as described previously [9].

2.2. Suramin

Suramin sodium salt was obtained from Sigma–Aldrich (St Louis, MO, USA). Stock solutions of 10 mM were

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0924-8579/$ – see front matter
doi:10.1016/j.ijantimicag.2007.11.001

prepared by dissolving the compound in RPMI medium and kept frozen at −20 °C. Final solutions were made in RPMI medium prior to each experiment.

2.3. Suramin treatment

After an infection period of 24 h, when all internalised trypomastigote forms were transformed into amastigote forms, host cells were rinsed twice and then incubated in RPMI-1640 medium supplemented with 2% foetal calf serum (FCS) containing 500 μM of suramin as previously described [9]. Trypomastigotes differentiated in the presence of suramin were named TDS, whilst control trypomastigotes were named WT. Since the population of intracellular parasites does not differentiate synchronously, amastigote forms could also be harvested from the cell culture either in control or suramin-treated host cells. Amastigotes obtained from suramin-treated LLC-MK_2 cells were named amastigotes grown with suramin (AGS).

2.4. Parasite smears

Control, TDS and AGS parasites were rinsed in phosphate-buffered saline (PBS), pH 7.2, spread onto a glass slide, air dried, dehydrated in methanol and stained with Giemsa. The cells were observed in a Zeiss Axioplan 2 light microscope and images were acquired with a Colour View XS digital video camera.

2.5. Parasite invasion assays

WT and TDS parasites were used for parasite invasion assays. Experiments were performed in 24-well plates containing 13 mm diameter, round, glass coverslips coated with 1.0×10^5 LLC-MK_2 cells, using a multiplicity of infection of 10. After a 3 h incubation period at 37 °C in a 5% CO_2 humidified atmosphere, extracellular parasites were removed by repeated washes and the cells were cultivated for an additional 3–4 days in RPMI-1640 containing 2% FCS. Following washes in PBS, the coverslips were fixed with Bouin's fixative, stained with Giemsa and mounted on glass slides with Permount (Fischer Scientific, New Jersey, USA). The percentage of infected cells was estimated by counting under a light microscope. All invasion assays were performed in triplicate, in at least three independent experiments and in the absence of suramin.

2.6. Transmission electron microscopy

Control and treated parasites were fixed in 4% formaldehyde, 2.5% glutaraldehyde, 0.1 M cacodylate buffer (pH 7.2) plus 5 mM calcium chloride for 1 h at room temperature. Cells were post-fixed, dehydrated and embedded as previously described [9]. Ultrathin sections obtained with a Reichert UltraCut S ultramicrotome were stained with uranyl acetate and lead citrate and then analysed and photographed in a FEI Morgagni F 268 transmission electron microscope.

3. Results and discussion

Suramin is currently used for the prophylactic treatment of African trypanosomiasis. Although suramin has not been used in Chagas disease, an in vivo study showed a significant suppressive action on *T. cruzi* infection in mice [10]. The mechanism of action of suramin is not completely understood but it is mainly attributed to the presence of six negative charges that interact with different serum and cell proteins [3].

In contrast to that described for African trypanosomes [11], only prolonged incubation of *T. cruzi* caused morphological and physiological changes in the parasite [9]. Since trypomastigotes cannot be maintained for long periods in axenic media at 37 °C without transforming into amastigotes, we developed a protocol in which suramin was introduced in the host cell culture medium 24 h after infection and maintained during completion of the parasite intracellular cell cycle [9]. The rationale for this experimental protocol is based on the fact that suramin is not only taken up by the cells [8,12] but also gains access to the cytoplasm where it could accumulate in a sufficient concentration to cause toxic effects to the amastigote and trypomastigote forms.

Morphological analysis of TDS and AGS parasites released 5–7 days post infection showed that they presented different grades of morphological changes (Figs. 1 and 2) mainly related to unfinished amastigote–trypomastigote differentiation and cytokinesis (Fig. 1B–F), as previously described for African trypanosomes, streptococci and clostridia, sea urchin eggs and young roots of *Vicia fava* [11]. Many binucleated forms were observed, suggesting that division of the cytoplasm had been inhibited before that of the nucleus. Epimastigote-like forms with round kinetoplasts (Fig. 1B and C) and trypomastigote forms with rod-like kinetoplasts (Fig. 1G) were observed. Most of the TDS and AGS parasites presented round kinetoplasts located side by side with the nucleus, suggesting that there was a delay in the process of kinetoplast migration from the anterior to the posterior end of the parasite (Figs. 1E–G and 2C). Changes in the nucleus shape were frequently observed (Fig. 2). In some parasites, an unfinished process of nucleus segregation was observed and the two new nuclei remained adhered to each other (Fig. 2E). Parasites in which nuclear division occurred but cell division was arrested presented two nuclei that were observed in different regions of the cell body (Figs. 1E,F and 2F). In contrast to most of the drugs used in *T. cruzi* chemotherapy, suramin did not cause changes in the mitochondrial structure (Fig. 2C).

Interestingly, the effects of suramin on *T. cruzi* described here were very similar to the alterations caused by blocking of the cytoskeleton associated with protein expression in