

RESEARCH NOTE

Ultrastructural Alterations Induced by Lithium Chloride in DNA-Containing Organelles of a Bat Trypanosome

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It is known that lithium ions induce several alterations in a number of biological systems. This monovalent cation stabilizes cytoskeletal elements, such as actin (R Colombo et al. 1991 *Biochem J* 274: 421-425) and microtubules (DE Burstein et al. 1985 *J Cell Biol* 101: 862-870), as well as interferes with DNA replication (EH Anwander et al. 1990 *Biopolymers* 29: 757-769) and inhibits cell cytokinesis (J Becerra & CL Encina 1987 *Experientia* 43: 1025-1027). Incubation of the trypanosomatid flagellate *Herpetomonas samuelpessoai* with lithium chloride resulted in inhibition of both cell proliferation and differentiation (CV Nakamura & AS Pinto 1989 *Parasitology* 99: 193-197). In this work we analyze the effects of lithium on the ultrastructure of a South American bat trypanosome.

The *Trypanosoma cruzi*-like strain M29-2 was isolated from a bat (*Phyllostomus hastatus*) col-

lected in Serrania, State of Minas Gerais, Brazil [AS Pinto et al. 1987 *Mem Inst Oswaldo Cruz* 82 (Suppl. I): 53], and has been characterized by zymodeme and schizodeme analysis (LFM Teixeira et al. 1993 *Parasitol Res* 79: 497-500). It has been shown that this trypanosome is non-infective to mice (S Hamanaka & AS Pinto 1993 *Rev Soc Bras Med Trop* 26: 225-230). Culture epimastigote forms have been maintained by weekly transfers at 28°C in brain-heart-infusion (BHI) medium (Difco, USA), supplemented with 10% fetal calf serum and 0.02% hemoglobin. Some cells were grown in BHI medium containing 50, 100 or 200 mM lithium chloride (Sigma Chem. Co., MO, USA). Cell counts were made, by light microscopy with a Neubauer chamber, at two-days intervals, up to the 12th day. The percent growth inhibition relative to control cultures was calculated at the 6th (middle log phase of growth) and 12th (stationary phase of growth) days post-inoculum. Epimastigotes from lithium-treated cultures were harvested at day 6 post-inoculum by centrifugation at 1,500g / 4°C, washed with phosphate buffered saline (PBS), pH 7.2, and then processed for routine transmission electron microscopy (TEM). To evaluate the effects of lithium chloride on the epimastigote-trypomastigote differentiation (metacyclogenesis), which occurs in old cultures, parasites were grown for 15 days in BHI medium and then 50, 100 or 200 mM LiCl was added to the medium. After six days, cells were collected, smears were stained with Giemsa, and the percent of cell differentiation inhibition relative to the control was calculated by light microscopy.

Cultivation of the parasites at 28°C in culture medium containing lithium chloride resulted in inhibition of both growth (Figs 1, 2) and differentiation (Fig. 3). Analysis of parasites grown in the presence of LiCl by TEM showed that Li⁺ induced severe alterations in the kinetoplast-DNA (k-DNA) and the nucleus. In affected cells, similar effects were observed with all concentrations (50, 100 and 200 mM) of the drug.

The k-DNA networks of the epimastigote forms lost their typical rod-shaped form (Fig. 4) and appeared as highly electron-dense bodies, presenting several morphological alterations, including incomplete fission (Fig. 5) and fragmentation in variable number of segments (Figs 6, 7). Most nuclei lost their round-shaped profile (Fig. 4) and appeared as irregular (Fig. 5), O-shaped or U-shaped structures. Masses of condensed chromatin were found apposed to the nuclear envelope. Both nuclear and k-DNA alterations were frequently observed in the same cell (Fig. 5).

Despite the alterations in the DNA-containing compartments, the parasites presented no signs of

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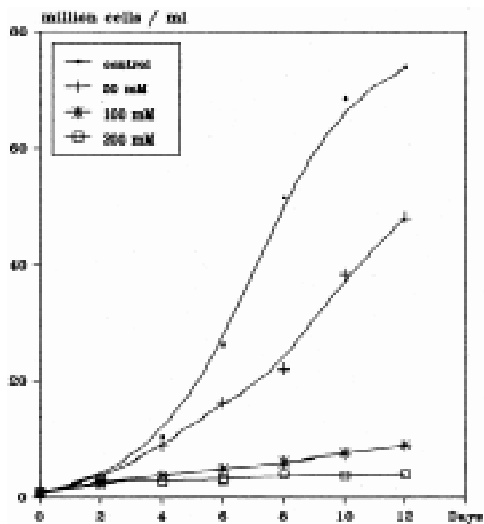


Fig. 1: growth curve of *Trypanosoma cruzi*-like parasites incubated at 28°C in brain-heart-infusion liquid culture medium in the presence or absence (control) of lithium chloride.

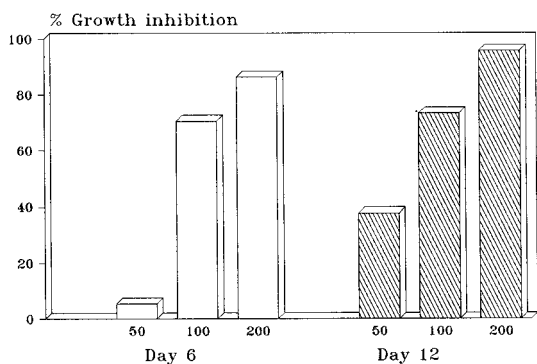


Fig. 2: percent growth inhibition of parasites incubated in culture medium containing 50, 100 or 200 mM LiCl, at days 6 and 12.

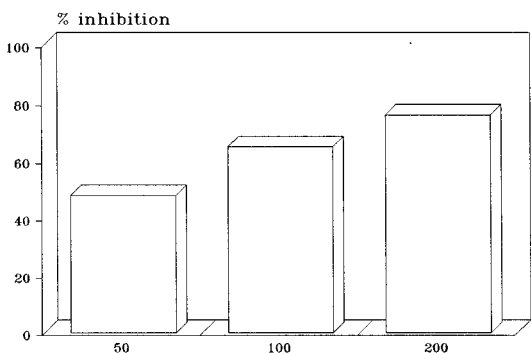


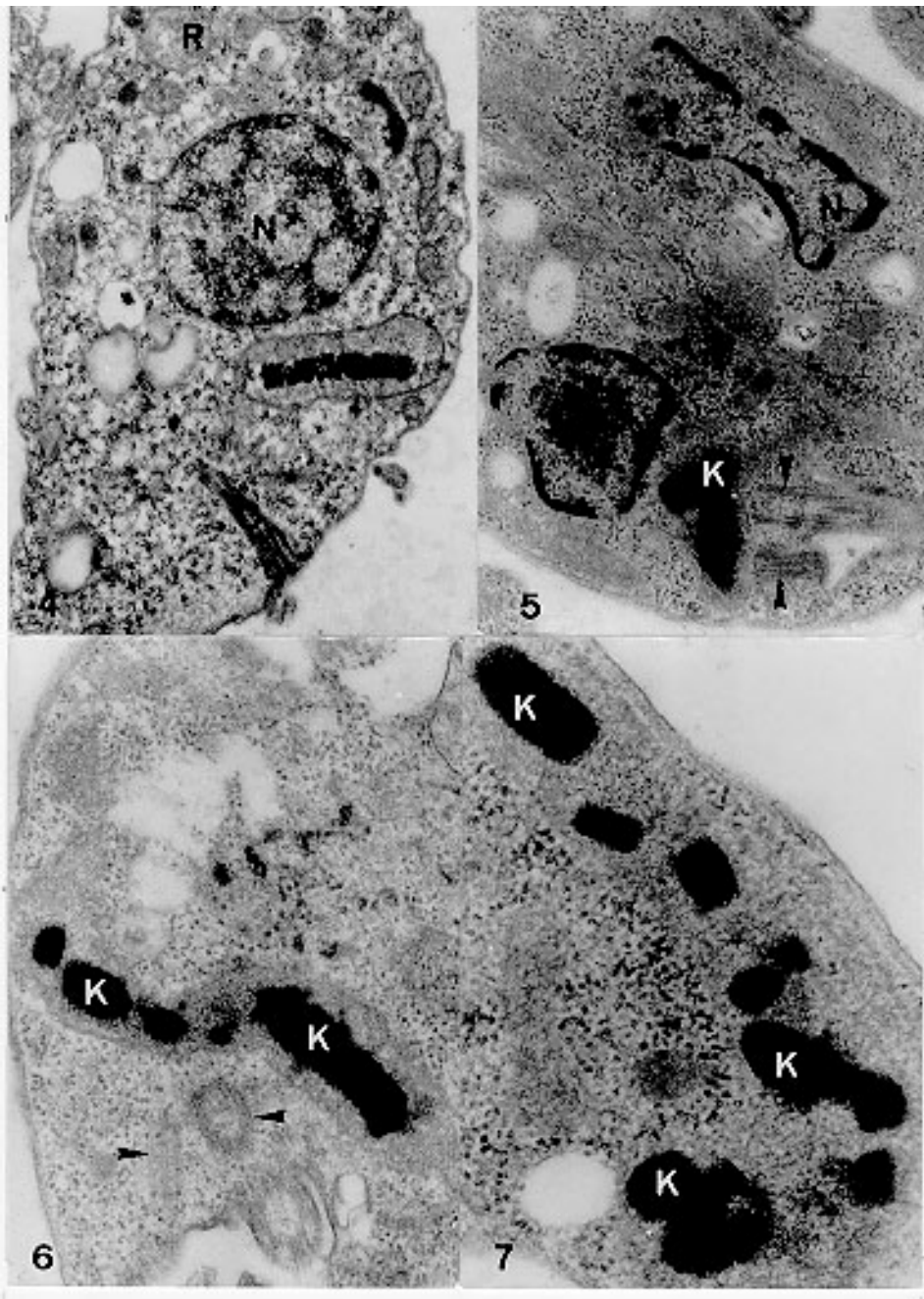
Fig. 3: percent of metacyclogenesis (epimastigote to trypomastigote differentiation) inhibition in 21-days-old cultures. Lithium (50, 100 or 200 mM) was added to 15 days-old cultures and then the cell differentiation was estimated six days thereafter and compared to that of control cultures.

cell degeneration, such as membrane disintegration or cytoplasmic swelling, or lysis. Most cell structures, such as subpellicular microtubules, flagella, Golgi complex and reservosomes appeared not to be affected by the drug, at least at the morphological level. Although most cells presented two basal bodies (Figs 5, 6), usually only a single flagellum could be observed, showing that cell division was blocked in an early stage. Preliminary biochemical analysis of the DNA by gel electrophoresis showed that DNA fragmentation (a hallmark of apoptosis) occurred, resulting in a ladder pattern on the gel (data not shown).

Our data suggest that lithium ions, under the growth conditions and concentrations used, act specifically on DNA or DNA-associated proteins of the parasites. Similar ultrastructural alterations in the k-DNA of epimastigotes of *T. cruzi* were observed after incubation of the cells with the drug hydroxystilbamidine (E Delain et al. 1971 *J Ultrastruct Res* 37: 200-217).

It is known that lithium ions bind to nucleic acid bases, leading to an increased H-bond stability (Anwander et al. *loc. cit.*), which would interfere with DNA replication. Therefore, the alterations observed in both the nucleus and the k-DNA could be explained as aborted attempts for duplication. Alternatively, it has been also described that lithium ions are powerful competitors of mono- and divalent cations, mainly sodium, magnesium and calcium (C Hori & T Oka 1979 *Proc Natl Acad Sci USA* 76: 2823-2827, K Ptashane et al. 1980 *J Cell Physiol* 103: 41-46). These cations are regulators of cell proliferation, acting on enzymes involved in initiation of DNA synthesis (H Rubin 1975 *Proc Natl Acad Sci USA* 72: 3551-3555, E Rosengurt & S Mendonza 1980 *Ann N Y Acad Sci* 339: 175-190). Therefore, enzymes such as DNA polymerase and DNA topoisomerases I and II could be the target for the lithium activity. It has been shown by *in situ* hybridization to the *Crithidia fasciculata* kinetoplast that these enzymes are components of peripheral complexes involved in k-DNA replication (M Ferguson et al. 1992 *Cell* 70: 621-629), and that *T. cruzi* proliferation and differentiation are blocked by topoisomerase inhibitors (M Gonzalez-Perdomo et al. 1990 *Antimicrob Agents Chemother* 34: 1707-1714).

Besides inhibiting either cell growth or differentiation, it has been recently shown that Li^+ induces apoptosis in rat immatures cerebellar granule cells (SR D'Mello et al. 1994 *Exp Cell Res* 211: 332-338). Apoptosis, or programmed cell death (PCD), is a process characterized by DNA fragmentation, detected either at the morphological or biochemical level (EM Johnson & TL Beckwerth 1993 *Ann Rev Neurosci* 16: 31-46). It has been re-



Effects of lithium chloride on the nuclear and kinetoplast-DNA (k-DNA) morphology. Fig. 4: untreated, control cell; six-day-old culture epimastigote form, showing the cytosome (C) and the rod-shaped k-DNA (K). The centrally located nucleus (N) is rounded, with electron-dense peripheral chromatin X 24,000. Figs 5-7: some aspects of k-DNA (K) anomalies induced by lithium chloride treatment. The same effects were observed in cells treated with either 50 (not shown), 100 (Figs 5, 7) or 200 (Fig. 6) mM LiCl. Alterations included incomplete fission (Fig. 5), as well as fragmentation of the DNA network in variable number of segments (Figs 6, 7). Fig. 5 also shows a dividing cell with both nuclear (N) and kinetoplast (K) alterations. Arrow heads: basal bodies. Fig. 5: X 37,000; Fig. 6: X 58,000; Fig. 7: X 94,500.

cently proposed that PCD occurs during the epimastigote-trypomastigote differentiation in *T. cruzi*, and may participate in the optimal adaptation of the parasite to its different hosts (JC Ameisen et al. 1995 *Cell Death Differentiation* 2: 285-300). Heat-shock induced apoptosis has been also produced in *Leishmania (L.) amazonensis* (MEC Moreira et al. 1996 *J Cell Physiol* 167: 305-313). Considering these data, and the results here presented on the effects of lithium ions on a digenetic

trypanosome, together with similar data obtained on an insect trypanosomatid (Nakamura & Pinto *loc. cit.*), it is possible that in these protozoa these effects involve a direct action of the ion on the DNA assembly, arresting cell division and leading to cell death by apoptosis. Thus, studies on lithium activity might be valuable in elucidating aspects of the regulation of cell proliferation and differentiation among trypanosomatid flagellates, at the cellular and molecular levels.