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ORIGINAL ARTICLE

In vitro EFFECTS OF SODIUM SELENITE SUPPLEMENTATION ON CELL VIABILITY OF DIFFERENT FORMS OF *Trypanosoma cruzi*

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ABSTRACT

Selenium is an essential trace element which, at adequate levels, presents different beneficial biological effects, such as cancer regression, tissue development and protection against oxidative damage. The positive effects of this element are related to the expression of selenoproteins and their ability to modulate the immune system and the oxidative stress response. In Chagas disease and sleeping sickness, selenium supplementation has shown blood parasitism reduction and the alleviation of specific aspects of the diseases, such as diminishing anemia in sleeping sickness or minimization of myocardial and right ventricular chamber damage in Chagas disease. Although the influence of selenium in trypanosomiasis has been investigated, the direct effects of sodium selenite supplementation on trypanosome cells are poorly understood. Treatment of *Trypanosoma cruzi* cultures with low selenium doses demonstrated different results, according to the parasite evolutive form analyzed. Epimastigote cultures supplemented with 100 nM of sodium selenite presented cell growth increment, which varies from 10 to 40% according to the parasite strain assayed. Selenium concentration around 600nM leads to a 30% increase in the amastigote form number, whereas, at the same dose, the mammal host cell presented no cellular growth alteration. For the bloodstream form, the results agree with the literature, and all sodium selenite concentrations tested, demonstrated a reduction in parasite viability. The data suggest that selenium supplementation, under specific conditions, could increase *T. cruzi* viability, demonstrating that a strategy for using selenium as an adjuvant in Chagas disease treatment requires additional experimentation.

KEY WORDS: Selenium; trypanosomiasis; Chagas disease; *Trypanosoma cruzi.*

INTRODUCTION

Two hundred years after the discovery of selenium (Se), the status of this element was altered from an exclusively toxic substance to an essential trace nutrient. Selenocysteine, the 21st amino acid, is the major biological form of selenium, and its incorporation in proteins, selenoproteins, requires a

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complex biochemical pathway. Selenoproteomes vary in size and composition in eukaryotes, and the highest number of selenoproteins is observed in fish bones, while parasites present a small number of members (Lobanov et al., 2006; Mariotti et al., 2012). Five selenoproteins have been indicated to be expressed in trypanosomes: the putative endoplasmic reticulum located SelenoK and SelenoT, and a third kinetoplastid specific selenoprotein SelenoTryp (Silva et al., 2014), and two hypothetical, selenoproteins exclusively in Leishmania (Cassago et al., 2006). Selenoproteins are involved in various biological processes such as protection against tumor progression, tissue and cell development, immunological system modulation and oxidative stress response (Carlson et al., 2009; Arbogast & Ferreiro, 2010; Schweizer & Fradejas-Villar, 2016). Deletion of components from the selenocysteine machinery was not able to impair trypanosome growth or infectivity (Aeby et al., 2009a; Aeby et al., 2009b; Bonilla et al., 2016). However, the absence of selenophosphate synthase, central in selenocysteine metabolism, decreased the ability of *Trypanosoma brucei* to respond to oxidative stress (Costa et al., 2011).

Some parasitic infirmities, such as Chagas disease (CD), caused by *Trypanosoma cruzi*, are designated as Neglected Tropical Diseases and are responsible for thousands of deaths or incapacitating conditions every year. About 7 million people present Chagas disease worldwide, mainly in the Americas where there is vector-borne as well as non-vector transmission, with outbreaks reported in Brazil, Venezuela, and Colombia (Franco-Paredes et al., 2020). Limited therapeutic strategies are the greatest challenge regarding CD. Nifurtimox and benznidazole are still the only commercially available drugs. However, although efficient during the early infection stages, the emergence of *T. cruzi* resistant strains and severe drug side effects illustrate the urgency for new pharmacological alternatives, either through optimization of existing drugs or the development of new compounds (Ribeiro et al., 2020). Selenium supplementation or its content in blood or tissues could alter the course of an infectious disease, including those caused by trypanosomatid species. Selenium-deficient mice presented a significantly lower immunological response to *T. musculi* infection (Ongele et al., 2002) and higher mortality to *T. cruzi* infection (de Souza et al., 2002). On the other hand, selenium supplementation contributes to host defense against human trypanosomiasis (da Silva et al., 2014). In *T. brucei* infected rats, sodium selenite supplementation presented conflicting effects. While intermediate doses reduced blood parasitism, higher doses led to its increase, which might be a consequence of host selenium toxicity. Positive effects of selenium include improved cell volume and higher hemoglobin concentration, indicating anemia reduction (Eze et al., 2013). Low selenium levels are correlated with cardiac insufficiency in chronic chagasic patients (Jelicks et al., 2011). Selenium supplementation in *T. cruzi* infected mice showed a dose-dependent blood parasitism reduction, myocardial and

right ventricular chamber damage prevention, and amelioration of intestinal symptoms (de Souza et al., 2003; de Souza et al., 2010a; de Souza et al., 2010b). Previous studies did not examine the effects of selenium supplementation directly on different *T. cruzi* developmental forms and the present work evaluated how the addition of sodium selenite interferes with epimastigote, trypomastigote and amastigote *in vitro* growth. Our findings show the opposite effects of selenium supplementation, depending on the dosage.

MATERIAL AND METHODS

T. cruzi *epimastigote culture and viability assays*

Four *T. cruzi* DTU (Discrete Typing Units) (Zingales et al., 2009) were used: *Triatoma lenti* isolate (DTU I) (Ribeiro et al., 2018) and strains Y (DTU II) (Silva & Nussenzweig, 1953); QMM-I (DTU V) (da Silva et al., 2016) and Tulahuen (DTU VI) (Pizzi et al., 1952). Strains were grown in liver infusion tryptose (LIT) medium at 26ºC with 10% inactivated FBS (Fetal Bovine Serum).

T. cruzi epimastigote viability was evaluated using CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay (Promega). Briefly, parasites were harvested during the exponential growth phase and settled (1 x 10^6 parasites/mL) into seven 96-well tissue culture plates (one to every 12 hours) following the addition of different concentrations of sodium selenite (0, 0.1, 1, 10, 50 and 100 μM). LIT alone and LIT supplemented with sodium selenite were used as negative controls.

The MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) based kit reagents were added to the cells, incubated for 1 hour at 26ºC followed by the determination of the absorbance at 595 nm. The cell viability results were normalized using the non-treated culture from biological triplicates.

To determine the growth rates of the parasite strains, 1x10^6 epimastigotes/mL were seeded in LIT medium, and kept at 26ºC. The number of epimastigotes was assessed in an hemocytometer each 24 hours.

T. cruzi *(Tulahuen strain) trypomastigote viability assays*

Trypomastigote cell viability was performed as da Silva et al. 2016, with modifications. Shortly, human fibroblast (Normal Neonatal Dermal Fibroblast) cell line, cultured in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum at 37°C and 5% CO_2 atmosphere, were infected with *T. cruzi* Tulahuen strain trypomastigotes at a 1:5 (cell: parasite) ratio. After approximately one week of infection, trypomastigote forms were collected and seeded (1 x 10^6 parasites/mL) in 96-well tissue culture plates and

different concentrations of sodium selenite (0, 0.1, 1, 10 and 100 μM) were added. The plates were incubated at 37ºC for 24, 48, 72 and 96 hours, and cell viability was evaluated with the CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay (Promega).

Human fibroblast and T. cruzi *amastigote viability assays.*

Amastigote viability assays were performed using a *T. cruzi* Tulahuen strain expressing β-galactosidase which catalyzes a colorimetric reaction utilizing β-D-galactopyranoside (CPRG) as substrate (Buckner et al., 1996). The experiments were performed as described by da Silva et al. (2016), with sodium selenite doses of 0, 100, 200, 300, 400, 500 and 600 nM. Control groups such as untreated infected fibroblast and uninfected fibroblast cells were added to the experimental set. Sodium selenite doses higher than 1 μM were toxic to human fibroblast, according to Hazane-Puch and coworkers (Hazane-Puch et al., 2014). Differently to the time-course experiments carried out on the epimastigote and trypomastigote forms, the amastigote viability was measured using an end-point assay. Therefore, the graphical form of expressing the results was adjusted to each analysis accordingly.

Human fibroblast viability assays were performed with 1 x 10^5 cells per well seeded into 96-well plates, incubated for 120 hours with the same selenium concentration of *T. cruzi* amastigote viability assays and analyzed using MTS test CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay (Promega). Cells without treatment were used as a negative control.

Statistical analysis

Statistical analysis were performed using two-tailed Student's t-test and differences were considered significant at $p \leq 0.05$ assessed in three independent experiments.

RESULTS

Low doses of selenium selenide increase epimastigote culture viability

Different concentrations of sodium selenite were added to different *T. cruzi* epimastigote strain cultures, from various DTUs (Figure 1). The purpose regarding testing several strains was due to characteristic *T. cruzi* genetic diversity, which reflects directly on drug sensitivity and/or resistance (Zingales, 2018). In all cultures tested, no growth effects were observed when 1μM of selenium was used. However, higher selenium concentrations (10, 50 and 100 μM) reduced the parasite viability drastically in a dose-dependent manner. Y strain (Figure 1B) was more susceptible to high doses of sodium

selenite, as it shows deleterious effects after 24 hours for 50 and 100 µM of sodium selenite, while *T. lenti* (Figure 1A), QMM-I (Figure 1C) and Tulahuen (Figure 1D) strains showed similar response only after 36 hours exposure. Strain growth curves (Figure 2A and 2B) demonstrated that the Y strain reaches a stationary phase at $2x10^7$ parasite/mL, while other strains reach the stationary phase at approximately $1x10^8$ parasite/mL. The lower parasite number in contact with an elevated selenium concentration could explain the more severe effects observed in the Y strain. On the other hand, selenium at a low concentration stimulates *T. cruzi* epimastigote culture growth, also *T. lenti* (Figure 1A), QMM-I (Figure 1C) and Tulahuen (Figure 1D) strains demonstrated a cell viability increment of approximately 40% when compared with the non-supplemented control. The Y strain presented a modest increase in growth, around 10% (Figure 1A).

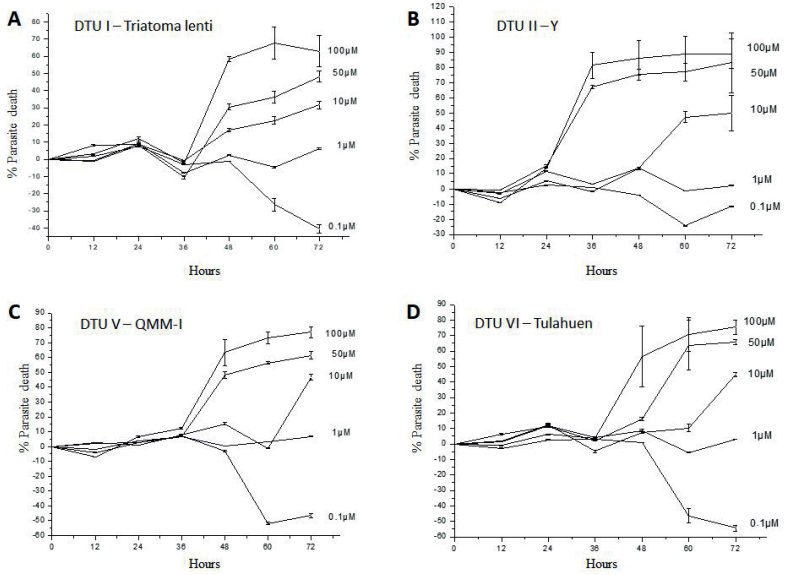

Figure 1. Effects of sodium selenite supplementation on cell viability of different strains of *Trypanosoma cruzi* epimastigote forms. Four *T. cruzi* DTU (Discrete Taxonomic Units) epimastigote strains: *Triatoma lenti* isolate (DTU I); Y (DTU II); QMM-I (DTU V) and Tulahuen (DTU VI) were grown in liver infusion tryptose (LIT) medium at 26ºC and cultures treated with different concentrations of sodium selenite (0, 0.1, 1, 10, 50 and 100 µM). Cell viability was evaluated using CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay (Promega) every 12 hours and the results expressed in the percentage of parasite death, normalized by the non-supplemented culture. Negative values ndicate that treated cultures grow more than non-supplemented controls.

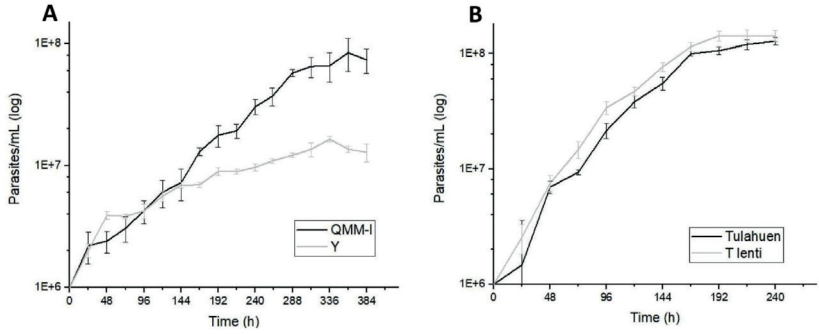

Figure 2. In vitro growth curves of *Trypanosoma cruzi* strains in LIT medium. Four *T. cruzi* DTU (Discrete Taxonomic Units) epimastigote strains: *Triatoma lenti* isolated (DTU I); Y (DTU II); QMM-I (DTU V) and Tulahuen (DTU VI) were grown at 28ºC in liver infusion tryptose (LIT) medium at $1x10^6$ epimastigotes/mL. Data was obtained, by counting epimastigotes in a hemocytometer chamber every 24 hours. A) Growth curves of QMM-I and Y strains; B) Growth curves of Tulahuen and *T. lenti* strains.

T. cruzi *Tulahuen strain trypomastigote form proved sensitive to all selenium concentrations tested.*

Only the Tulahuen strain was tested in trypomastigote and amastigote cell viability measurements. Among *T. cruzi* strains assayed, in the epimastigote form, the Tulahuen, QMM-I and *T. lenti* presented similar selenium sensitivity, which justified the use of only one strain in subsequent assays. However, the Y strain was more sensitive to elevated selenium doses and presented a less significant viability increment at a low sodium selenite concentration. Nevertheless, the Tulahuen strain expresses the β-galactosidase enzyme, allowing a colorimetric assay that presents higher sensitivity and reproducibility. Attempts to transfect the plasmid pBS:CL-Neo-01/BC-LacZ-10 (Buckner et al. 1996), for β-galactosidase expression, in other strains did not result in the selection of stable cell lines (data not shown). Moreover, the analysis of several forms of *T. cruzi* may exhibit different results in the insect and the mammal forms of the parasite.

Selenium supplementation on trypomastigote forms reduced cell viability in all concentrations tested (Figue 3A), although the parasite viability difference was not statistically significant. For 0.1 µM of sodium selenite parasite death was around 20%, while with other concentrations cell viability decreased to approximately 40%. The data agree with the blood parasitism decrease observed in *T. brucei* (Eze et al., 2013) and *T. cruzi* (Davis et al., 1998) experimentally infected mice.

Selenium supplementation increases T. cruzi *amastigote Tulahuen strain viability*

Initially, the impact of sodium selenite doses on human fibroblast viability was verified. If a concentration tested decreased or increased the host cell growth/viability, this could produce false results related to parasite amastigote form development. Therefore, selenium concentrations varying from 100 to 600 nM did not alter fibroblast viability and values detected for each dose were around 100%, as observed for untreated cells (Figure 3C). Treatment with different concentrations of selenium increased, in a dose-dependent manner, the number of amastigote forms in infected fibroblast cells (Figure 3B), reaching a 30% increase at 600 nM of sodium selenite (Figure 3B).

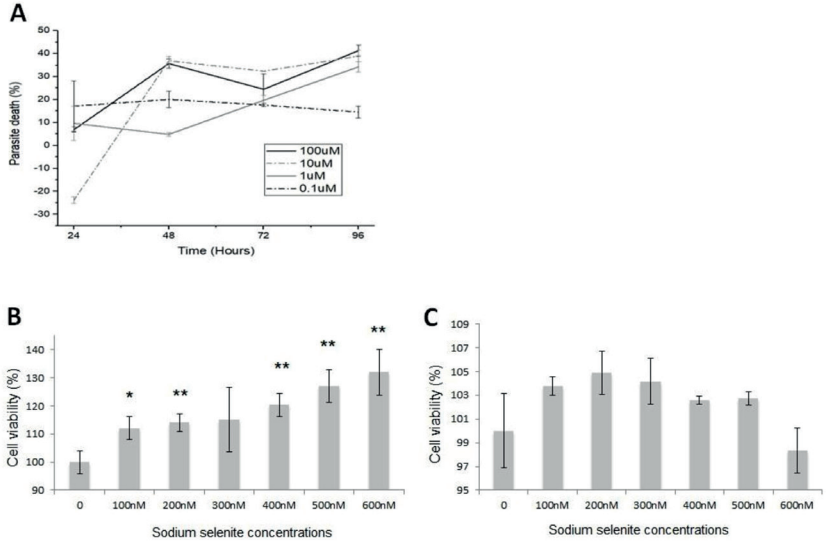

Figure 3. Effects of sodium selenite supplementation on different forms of *Trypanosoma cruzi* tulahuen strain and viability on fibroblast cells. A) Effects of sodium selenite supplementation on *Trypanosoma cruzi* trypomastigote viability (strain tulahuen). Cells were incubated with 0, 0.1, 1, 10 and 100 µM of selenium selenite and viability was performed using the CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay (Promega). Results were expressed in absorbance at 595nm. B) Amastigote viability assay measured by β-galactosidase colorimetric assay of Tulahuen *T. cruzi* strain treated with selenium concentration varying from 0 to 600 nm. C) Fibroblast viability assay evaluated by CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay (Promega), incubated with the same doses as in the amastigote viability assay.

DISCUSSION

Selenium is an essential element to various cell lines, and its effects are directly related to dose administration. In *T. cruzi* culture, low doses of sodium selenite increased cell viability of epimastigote and amastigotes forms, however, higher concentrations strongly reduced the growth of different strains of *T. cruzi* (epimastigote). For trypomastigote form, any concentration tested led to a decrease in viability, although not statistically significant. However, measuring the selenium content in a culture medium is not simple, due in part, to the complexity of the possible selenium compounds, in special organic forms, such as selenomethionine, selenocysteine, selenoglutathione, and others. Bryan and co-workers analyzed the composition of seven commercial Fetal Calf Sera (FCS) and observed that the selenium concentration varies from 400 to 1000 ppb (parts per billion) when 20% is added to the medium (Bryan et al., 2011). The smallest concentration used in epimastigote medium, 0,1 μM of sodium selenite, represents around 17.000 ppb, and in amastigote cultures, the values vary from 17.000 to 102.000 ppb. Therefore, supplementation greatly increased the amount of selenium in the medium and the difference observed in the effective concentration is probably due to the higher number of selenoproteins present in mammal cells, which require more available selenium.

Selenium compounds present a dual effect. At high concentrations they induce oxidative stress in mammal cells, with potential single-strand DNA breaks due to the formation of reactive oxygen species (ROS) (Zhou et al., 2003). Selenite-induced oxidative stress led to the decline of the intracellular reduced glutathione and the increase of oxidized glutathione, which is a product of the non-enzymatic selenium metabolism reaction. Also oxidative stress attenuation by superoxide dismutase, catalase, and deferoxamine reinforce the role of ROS in the DNA break and the apoptosis process (Shen et al., 1999). Trypanosomatids possess an entire repertoire of specific enzymes involved in oxidative stress defense, although it is still unclear how this system is affected by high selenium concentrations.

Selenium supplementation showed protective effects against various harmful factors such as severe drug side effects, heavy metal toxicity, protection against pesticide carcinogenic effect and others (Kiełczykowska et al., 2018). Positive effects of sodium selenite were observed at a late growth phase in *T. cruzi* epimastigote or amastigote form suggesting that selenium helps reduce damage caused by oxidative substances, which accumulate with parasite growth. Selenium supplementation in pregnant Wistar rats infected with *T. cruzi* reduced blood parasitism and pro-inflammatory cytokines while parameters such as fetus and placenta morphology and number of amastigote nests in the cardiac tissue remained unaltered. However, selenium supplementation promoted the extensive proliferation of amastigote nests on

placenta tissue and authors attribute these effects to the altered balance in pro-inflammatory cytokines, creating a scenario that contributes to *T. cruzi* propagation (de Freitas et al., 2018).

Our findings indicate the direct effect of selenium on parasite forms, however, it is premature to attribute the results observed exclusively to parasite selenoprotein activity. Immune system cells, such as macrophages, respond to selenium intake, resulting in inflammatory process regulation, which contributes to a suitable response to several injuries (Carlson et al., 2010; Bi et al., 2016). However, other tissues, such as those presenting fibroblast as a component, may respond differently, leading to the parasite infection and proliferation. Supplementation with non-toxic doses of sodium selenite confirmed the antioxidant properties of selenium, demonstrated by the increase of GPX1 and SEPW1 activity in human fibroblast (Hazane-Puch et al. 2014). *T. cruzi* infected mice, overexpressing GPX1, treated with a subunit vaccine exhibited additional benefits such as a reduced parasite burden, decreased inflammatory/oxidative stress and cardiac remodeling in comparison with wild animals (Gupta et al., 2015). Additional experiments are necessary to improve the information available about selenium metabolism in trypanosomes and clarify deleterious and/or beneficial actions of this compound in different trypanosomiases.

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CONFLICT OF INTEREST

The authors state that the content of this article presents no conflicts of interest.

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