**Supplementary material**

**Tamoxifen alters cell membrane properties in *Leishmania amazonensis* promastigotes**

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*Supplementary Video 1*

*L. amazonensis* promastigotes incubated in culture media in 24 well plates with tamoxifen 10 µM.

*Supplementary Video 2*

*L. amazonensis* promastigotes incubated in culture media in 24 well plates with tamoxifen 20 µM.

*Analysis of cytosolic free Ca2+ concentration ([Ca2+]c)*

Intracellular free Ca2+ concentrations were measured using the Ca2+ indicator Fluo-4/AM (Molecular Probes) as described previously (Dolai *et al.*, 2011), with minor modifications. Logarithmic-phase *L. amazonensis* promastigotes (2×107/mL) were loaded with 5 μM Fluo-4/AM in PBS Ca2+ free for 15 min at 25 °C. Incubation with EGTA (Sigma-Aldrich), BAPTA-AM (Molecular Probes) or both calcium chelators were carried out during 15 min prior to Fluo-4/AM loading (Nicolao *et al.*, 2014). In some experiments, 500 µM CaCl2 was added to the buffer for assays with calculated calcium concentrations. In these experiments, Ca2+ was buffered with 5 mM EGTA.

After incubation, cells were added to black polystyrene 96-well microplates in a final volume of 100 μL per well. The plate was incubated at 25 oC and fluorescence was recorded (λex = 485 nm; λem = 530-10 nm) every 12 sec in a microplate reader (POLARstar Omega, BMG Labtech). After signal stabilization, 5, 10 or 40 μM tamoxifen was added. Ionomycin (Sigma-Aldrich) at the same concentrations was used as a positive control. Untreated parasites and parasites incubated with the highest volume of diluent (DMSO) were used as negative controls. To test if tamoxifen interfered in Fluo-4 fluorescence, the drug was assayed in the absence of cells.

A dose-dependent increase in the indicator’s fluorescence was noted upon addition of tamoxifen to the cells (Supp. Figure 1), an effect observed with growing intensity over time. After 30 min incubation, cells treated with 5, 10 and 40 µM tamoxifen showed 1.8, 2.8 and 4.7-fold increase in Fluo-4 fluorescence, respectively. When compared with untreated parasites, 5 µM tamoxifen resulted in a 2.6-fold increase in Fluo-4 fluorescence after 60 minutes of incubation.

The ionophore ionomycin used as positive control also displayed a dose-dependent effect (Supp. Figure 1B). In the absence of cells, either the diluent (DMSO), or the drugs tamoxifen and ionomycin in the same concentrations used in the experiments described above resulted in no changes in Fluo-4/AM fluorescence (data not shown).

In order to investigate the role of intracellular stores in the rise of [Ca2+]c, the cell-permeant Ca2+ chelator BAPTA-AM was added to the parasites before tamoxifen treatment. A dose response effect was observed upon addition of BAPTA-AM, which inhibited the increase in fluorescence immediately after its addition (Supp. Figure 1C).

A partial inhibition of the increase in [Ca2+]c triggered by tamoxifen was noted when the assay was performed in the presence of known concentrations of extracellular Ca2+ chelated with 5 mM EGTA (Supp. Figure 1D). There was a partial inhibition of the increase in fluorescence, indicating that movement of Ca2+ from the extracellular environment through the membrane at later time points was also occurring. When both external and internal Ca2+ pools were chelated through incubation in 1 mM EGTA plus 50 µM BAPTA-AM, the increase in [Ca2+]c triggered by tamoxifen was totally inhibited (Supp. Figure 1D), indicating that tamoxifen was able to release Ca2+ from intracellular stores in *Leishmania* promastigotes.



Supplementary Figure 1. Measurement of [Ca2+]c in tamoxifen-treated promastigotes.*L. amazonensis* promastigotes were loaded with 5 μM Fluo-4/AM and fluorescence was recorded continuously (λex = 485 nm excitation; λem = 530-10 nm). Tamoxifen treatment showed an increase in free [Ca2+]c in a dose dependent manner (A). The ionophore ionomycin was used as positive control (B). Parasites treated with increasing concentrations of BAPTA followed by incubation with 5 µM tamoxifen displayed a dose-dependent inhibition of the increase in fluorescence (C). Parasites incubated in PBS with 500 µM CaCl2 were pretreated for 15 min with 5 mM EGTA and/or 50 µM BAPTA-AM before addition of 5 µM tamoxifen (D). The arrow indicates when tamoxifen or ionomycin were added. Traces are from one experiment representative of three independent experiments. NT: untreated parasites; T: tamoxifen (5, 10 or 40 μM); I: ionomycin (5, 10 or 40 μM); E5 (EGTA 5 mM); B (BAPTA-AM 25, 50 or 100 µM).

**References**

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