TITLE: CHANGES IN PARASITOPHOROUS VACUOLE FORMATION DURING INTERACTION BETWEEN MACROPHAGES AND BROMOENOL LACTONE TREATED *LEISHMANIA AMAZONENSIS*.

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ABSTRACT:
Leishmaniasis is a health problem in 98 countries in four continents, and World Health Organization estimates about 350 million people are under risk of infection, with around 2 million new cases/year. *Leishmania amazonensis* is the causative agent of cutaneous, diffuse cutaneous and disseminated cutaneous leishmaniasis. Parasites modulate its virulence factors via endo- and exocytosis mechanisms to improve infection thus, these mechanisms become important targets to modulate the process of parasite infection and survival in the host. Previous results from our group evidenced that the inhibition of *L. amazonensis* promastigotes enzyme Ca^{+2}-independent phospholipase A2 with bromoenol lactone (BEL), a specific and irreversible inhibitor, decreased parasite internalization by peritoneal murine macrophages. Moreover, the characteristic large parasitophorous vacuole (PV) formed by this species was significantly decreased after macrophage interaction with BEL-treated promastigotes. Thus, the aim of this study is to compare PVs formed by untreated and BEL-treated *L. amazonensis* in murine peritoneal macrophages, analyzing vacuole size, pH, lipid content and markers of endocytic compartments. Our results confirmed the changes in the macrophage vacuole morphology formed after phagocytosis of BEL treated-promastigotes. This vacuole presents reduced size, often appearing very tight around parasites. Quantification of vacuole size evidenced that BEL treated-promastigotes showed a higher proportion of small vacuoles (56%) compared with the PVs formed after phagocytosis of untreated promastigotes (13%). Furthermore, a smaller number of parasites were observed inside PVs containing BEL-treated promastigotes than in PVs formed by untreated parasites. No difference was observed between the pH of both PVs, using acridine orange. The investigation of compartments fusion between lysosomes and phagosomes using fluorescent beads, showed that the fusion occur equally in macrophages infected by BEL-treated and untreated parasites. Also, it was observed that Rab(+) vesicles fuse equally to PVs of both BEL-treated and untreated-*L. amazonensis*. Our initial results suggest that the smaller PV formed after BEL-treated promastigotes was not due to inhibition of lysosomes fusion to the PVs or to pH differences.

Keywords: Leishmania amazonensis, Bromoenol lactone, parasitophorous vacuole, machophages.

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