Highly diluted natural complex (M2) effects on leishmanias

Katia Fialho do Nascimento, Jenifer Pendiuk Gonçalves, Maurício Baum, Izanara Pritsch, Magda Clara Vieira da Costa Ribeiro, Edilene Alcântara de Castro, Carolina Camargo de Oliveira, Dorly de Freitas Buchi

Federal University of Paraná, Curitiba, Paraná, Brazil

Introduction: Leishmaniosis is regarded as a serious public health issue either by its magnitude, morbidity or mortality. Depending on *Leishmania* species disease ranges from cutaneous, which is relatively confined and controlled, until a progressive and fatal visceral disease [4]. In order to complete their lifecycle *Leishmania* undergo transformations in the vector digestive tract to get to metacyclic infective form [3,5]. Those include secretion of the “promastigote secretory gel” (PSG) that protect parasites from digestive enzymes. PSG acts on survival and colonization of parasites inside the vector, on transmission and infection development in mammals, and also facilitates and increases transmission [1,2]. Drugs used for leishmaniosis treatment show high toxicity and several side effects, leading patients to quit the treatment and consequently generating resistant strains. The search for new therapeutic approaches is considered a strategic research priority by the World Health Organization. Highly diluted natural products show efficacy in modifying immunological response by stimulating the immune system through macrophages activation, then favoring the organism in many pathological conditions. **Aim:** To assess the direct action of M2 treatment on promastigotes of different *Leishmania* species. **Methods:** 3x10⁶ were cultured in the presence of 20% of M2 plus booster 1% doses every 24h for up to 96h at 25°C in humidified incubator and then submitted to assays for determining mitochondrial activity by MTT (5mg/mL), cell proliferation through cell counting on Neubauer chamber and rosettes formation (around PSG) by light microscopy. Results of 3 independent experiments were statistically analyzed using t-test. **Results:** M2 treatment changed mitochondrial metabolic activity in all tested *Leishmania* strains. *L. amazonensis* proliferation decreased after 96h treatment and likewise the rosettes formation (total, closed and open ones). **Discussion:** Our data show that *Leishmania* cultures treated with M2 present decreased number of rosettes and these rosettes may be slowing the production of PSG, that is typically synthesized by *Leishmania* during metacyclic infective phase. Rosettes are also a place for fusion between 2 or more leishmanias, a process that involves nuclear and kinetoplast genetic material exchange. **Conclusion:** M2 acts on *L. amazonensis* promastigote forms by reducing the total number of rosettes (the same for open and closed rosettes) that are related to infective form of promastigotes, which produce PSG while on rosettes. This result suggests that M2 treatment is capable of decreasing *Leishmania* infectivity. Although our results are preliminary, these changes open new perspectives for the disease treatment and/or prevention using M2.

Keywords: promastigote secretory gel, infectivity, proliferation, rosettes.

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Figure 1. M2 treatment changed mitochondrial metabolic activity, cell proliferation and rosettes formation in *Leishmania*. Graphs A and B represent mitochondrial activity of the strains *L. amazonensis*, *L. braziliensis*, Cur 453 (*L. amazonensis*) and Cur 454 (*L. braziliensis*) treated with M2 for 48 and 72h. Graph C shows cell proliferation of *L. amazonensis*. The images illustrate what we considered as closed (D) and open rosettes (E), which were stained using May Grünwald and Giemsa.

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Correspondence author: Dorly de Freitas Buchi, buchi@ufpr.br dorlybuch@gmail.com

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