GENE SILENCING IN *Giardia lamblia* THROUGH RNA INTERFERENCE, A STRATEGY FOR THE DESIGN OF SPECIFIC DRUG

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Introduction. *Giardia lamblia*, a unicellular flagellate protozoan is responsible of giardiasis; this intestinal parasite has worldwide distribution and affect human and several mammals (Adam, 2001). Giardiasis is a principal public health problem, children and immunocompromised are the main affected groups. An alternative to generate specific drugs for its treatment is the analysis of metabolic pathways of gene by RNA interference.

In this context, our objective was to design a vector to generate the gene silencing in Giardia, taking as a model system for studying the gene of the NADH Oxidase (NADHox)

Methods. We report the construction of a vector with two promoters positioned faced each and oriented at opposite direction between them (pTub::pGdh); such distribution allows the synthesis of siRNAs for gene silence. We introduced by electroporation the integrative vector pNADH_egfp-RNAi to *G. lamblia* trophozoites; the vector contains the fragment of 412 pb from NADHox gene, plus the puromycin resistance cassette for selection in Giardia (Davis- Hayman y Nash, 2002). The kinetics for quantifying growth, enzyme activity and gene expression were carried out in TYI with sampling times of every 12 h.

Results. The correct integration of the pTub-NADH fragment-pGdh silencing cassette was analyzed by PCR, the protein expression of *egfp* was also determined by RT-PCR and confocal microscopy.

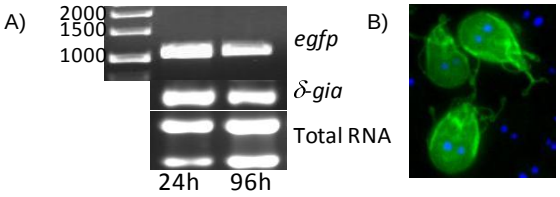

Fig. 1. (A) RT-PCR of the gene and (B) the confocal microscopy of the green fluorescent protein. δ-Gia gene housekeeping

When was inhibited NADHox, affection of how important its growth from the 24 hours of cultivation (Fig 2A). However, apparently the enzyme activity was not affected, but until after the 60 h (Fig. 2B).

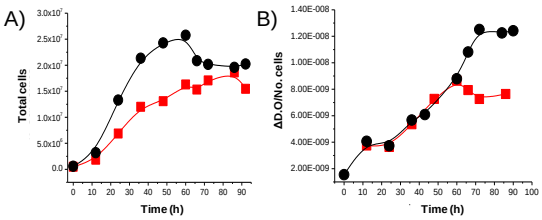

Fig. 2. (A) Kinetics of growth and enzyme activity of wild-type strain (•) and the transform (■).

Its corresponding RNAm was determined by RT-PCR. The data showed that at 24 and 48 hours of cell growth, there were no changes in levels of RNAm from NADHox (Fig. 3B); however, at 72 and 96 hours an important reduction of this RNAm was detected, Similar to that observed in the densitometry done.

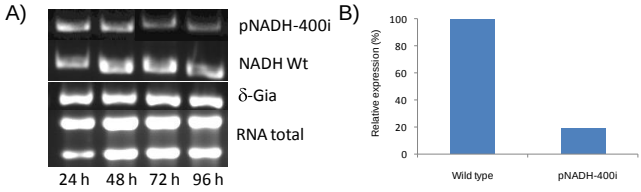

Fig. 3 (A) RT-PCR obtained from the kinetic. B) Densitometry of the wild-type strain and silenced to the 72 h of cultivation.

Conclusions. The vector designed, caused gene silencing of the NADHox, affecting the growth of giardia and expression of gene, and considered as a possible target for design of specific drug.

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References.

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