Metacyclogenesis in vitro: do all *Trypanosoma cruzi* strains behave the same?

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Chagas disease is caused by *Trypanosoma cruzi*, and it is a parasitic infection that affects millions of people worldwide. Metacyclogenesis is the process by which non-infective forms (epimastigotes) are transformed into infective forms (metacyclic trypomastigotes), occurring in vivo at the triatomine vector digestive system. In vitro metacyclogenesis can be conducted submitting epimastigote cells to nutritional stress in triatomine artificial urine (TAU) medium and further incubation in TAU supplemented with amino acids and glucose (TAU3AAG). This process facilitates several studies, such as differential gene expression, molecular mechanisms and metabolic pathways. However, previous studies related that not all *T. cruzi* strains behave the same and there are differences related to the metacyclic trypomastigotes rate obtained by this in vitro differentiation. The aim of this study is to compare the number of initial epimastigotes with the final number of metacyclic trypomastigotes obtained by metacyclogenesis in vitro for different *T. cruzi* strains. We conducted in vitro metacyclogenesis using TAU/TAU3AAG mediums for Dm28c (TcI), RN1 (TcII) and CL (TcVI) *T. cruzi* strains. Epimastigotes and metacyclic trypomastigotes were quantified using a Neubauer chamber and further Leishman coloration was conducted to establish the differentiation rate. We have identified that there are significant differences for in vitro differentiation among each *T. cruzi* strain. Some strains can present higher metacyclic forms number at the end of the process. Moreover, we have found that these differences can have a huge impact in the experiments, when the researcher chooses to work with a strain that has difficult at in vitro differentiation and a high number of metacyclic forms is needed. Our analysis could eventually help researches to perform their experimental design, making the right strain choice for each specific study. Finally, our results open the perspective of new biological and biochemical studies to better understand the differences observed.

Key words: *Trypanosoma cruzi*, metacyclogenesis, in vitro differentiation.