A NEW FUNCTIONAL MULTIPARAMETRIC FLOW CYTOMETRIC ASSAY USING WHOLE HUMAN BLOOD WITH COMMITMENT TO REDUCE ANIMAL USE IN DRUG DISCOVERY PROCESS FOR NEGLECTED DISEASES

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The top 13 neglected diseases worldwide include the Trypanosomatids such as leishmaniasis and Chagas Diseases. New drugs to treat these diseases are urgently needed due to their high toxicity, resistance and low cure efficacy. In drug discovery development, the in vitro assays validated for early stage screening aimed at identifymolecules with eficacty, filtering out those molecules with higher potential for toxicity and contribute to replace or reduce the use of in vivo tests. In this context, at the pipeline to discover drugs against Trypanosomatids, the in vitro assays used include the use of free parasites, their intracellular forms or the combination assays. In order to contribute with in vitro assays to evaluate potential anti-Trypanosomatidae drugs, our group has developed a new quantitative and functional predictive assay to support the early identification of promising drug candidates and contribute on reduction of animal studies . This preclinic ex-vivo assay allows the quantitative and simultaneous evaluation of anti-trypanosomatidae, cytotoxic and immunomodulatory activities of substances using flow cytometry. Two drugs used in clinic to were evaluated against leishmania (Amphotericin B) and T. cruzi (Ravoconazole) using human whole blood that mimetize infection in vivo. Multiparametric analysis by FACS was done through labelling of parasite and leukocytes with different fluorochromes after erithrocytes lysis to quantify: (1) anti-trypanosomatidae effect through quantification of parasite internalization by monocytes and neutrophils (trypanosomatidal effect); (2)quantification of cytokines inside of lymphocytes and phagocytes or (3) at supernadant of culture to evaluate immunomodulatory effect and cytotoxicity against leukocytes using viability dyes. The assay could predict the anti-trypanosomatidae activity, the immunomodulatory effect and toxicity of both drugs as observed in vivo. Moreover, it also presents as a alternative opportunity to animal testing to improve success rates to discover new drugs to neglected diseases coupled with a strong commitment to the 3Rs.

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