ABSTRACT

Background: Approximately 6 to 7 million people are infected by Chagas disease in the world, mostly in Latin America. However, due to its neglected condition by the governments and pharmaceutical industry, the advances for finding new treatments are slow and heavily dependent on academia-based drug development. Because of the limitations in efficacy of current Chagas disease treatment, the severe toxicity caused by the available drugs Benznidazole and Nifurtimox as well as the emerging resistant parasites to those drugs, it is imperative to find new options for the treatment of this infectious disease. *Trypanosoma cruzi* acetate:succinate CoA-transferase (TcASCT) is an enzyme catalyzing the conversion of acetyl-CoA and succinate to succinyl-CoA and acetate, which are important metabolite throughout the life cycle of this parasite. The reaction of ASCT is coupled to succinyl-CoA synthase that produces ATP using succinyl-CoA and re-generates succinate in a cycle known as the ASCT/SCS cycle. The ASCT is not conserved in mammalian host. Such exclusively conservation in the parasite makes this enzyme a potential candidate for development of new anti-trypanosomal drugs.

Objective: The main objectives of this study were to carry out the biochemical characterization of TcASCT and performing a primary screening for finding potential inhibitory compounds of the TcASCT/SCS cycle.

Method: I have purified the recombinant TcASCT from *E. coli* BL21 Star™ (DE3), biochemically characterized using the *in vitro* reconstructed TcASCT/SCS cycle and

developed a high-throughput screening (HTS) system, which was used to screen 40,640 compounds from Kyoto University chemically defined library with excellent performance.

Result: I could purified to homogeneity the recombinant TcASCT with a specific activity of 108 µmol/min/mg representing 34-fold purification compared to the lysate fraction and characterized it by *in vitro* reconstitution of the ASCT/SCS cycle. In the HTS performed for ASCT, I successfully identified 6 hits (more than 50% inhibition at 4 µM) in the primary screening and 24 compounds showing moderate hits (in the range of 20-50% inhibition). Reproducibility, specificity and anti-trypanosomal activity of identified hits are currently ongoing.

Conclusion: In this report, I biochemically characterized the ASCT from *T. cruzi* for the first time and developed a HTS system, identifying six TcASCT inhibitor candidates from Kyoto University library. The analysis of specificity toward SCS and potential inhibition of human SCOT, followed by toxicity and anti-trypanosomal activity will be addressed in next steps. Such assays are essential for the selection and prioritization of the hits that will be advanced to the hit-to-lead campaign in the future

Keywords: *Trypanosoma cruzi*, energy metabolism, acetate:succinate CoA-transferase, biochemical characterization, high-throughput screening.