

Protocol for drug testing in *T. cruzi* acute phase in infected mice

Experimental animals

Female Balb/c mice, 5 weeks old (20 - 25g). Groups of five mice are divided in control (vehicle treated) and the different groups of drug treatments.

Transgenic parasite line

The transgenic *T. cruzi* Brazil strain expressing firefly luciferase is used (Andriani et al., *PLoS Negl Trop Dis.* 2011 Aug;5(8):e1298. PMID: 21912715). The parasite was transfected with pTREX-luc and has been selected by growing in medium containing G418.

In vivo development of blood stages + Drug treatments

Groups of 5 mice are infected via i.p. injection with 10^6 *T. cruzi*-Luc trypomastigotes forms harvested from the supernatant of infected LLC-Mk2 cells.

Three days after infection the mice were anesthetized by inhalation of isoflurane (controlled flow of 1.5% isoflurane in air was administered through a nose cone via a gas anesthesia system). Mice were injected with 150 mg/kg of D-Luciferin Potassium-salt (Goldbio) dissolved in PBS 20mg/ml. Mice were imaged 5 to 10 min after injection of luciferin with an IVIS 100 (Xenogen, Alameda, CA) and the data acquisition and analysis were performed with the software LivingImage (Xenogen). One day later (4 days after infection) treatment with compounds at the desired dose or vehicle control (2% of methylcellulose + 0,5% Tween 80) is started by i.p. injection or oral gavage. After treatment, mice were imaged again after anesthesia and injection of luciferin as described above. The ratio of parasite levels is calculated for each animal dividing the luciferase signal on day 9 (after 5 days of treatment) or day 14 (after 10 days of treatment) by the luciferase signal on day 3 (before treatment starts on day 4). Benznidazole can be used as a control drug at the dose of 15 mg/kg/day i.p. or 30 mg/kg/day oral for 10 days.

