NYU School of Medicine
Department of Microbiology

Ana Rodriguez, Ph.D.
Associate Professor
Ana.Rodriguez@nyumc.org

Protocol for drug testing in *P. berghei* liver stage infected mice

Experimental animals

Female Swiss Webster mice, weighing 25 to 30 g. Groups of five mice are divided in control (vehicle treated) and the different groups of drug treatments.

Transgenic parasite line

The transgenic *P. berghei* line 676m1cl1 line (PbGFP-Luccon) is used (Franke-Fayard et al., 2005. PNAS 102(32):11468-73. PMID:16051702). It expresses a fusion GFP (mutant 3) and firefly luciferase (LucIAV) and has been generated in the reference clone of ANKA strain cl15cy1. Parasites of line 676m1cl1 contain the PbGFP-Luc gene fusion stably integrated s a single copy gene by double cross over recombination into the 230p locus and the reporter gene is under control of the constitutive eef1aα promoter. This line has been selected by FACS-sorting of GFP-expressing parasites and therefore does not contain a drug-selectable marker.

In vivo development of liver stages + Drug treatments

Groups of 5 mice are infected by being exposed for 15-20 min to the bites of 10 to 50 mosquitoes infected with *P. berghei*-Luccon. Mice are anesthetized by i.p. injection of 300 mg/kg of Xylazine and 3500 mg/kg of Ketamine before exposure to mosquitoes. Mice are treated on day -1, day 0 and day 1 after infection. The control group is treated with vehicle, while the test groups are treated i.p. with compounds at the determined mg/kg body weight in vehicle. As control, a group of mice will be treated with Primaquine at 30 mg/kg/day for i.p. and 45 mg/kg/day orally. Compounds can be administered i.p., i.v. or through oral gavage. All groups are imaged 40 to 42 h after infection (never longer than 42 h).

Live Imaging

Two days after infection the mice are anesthetized by inhalation of isofluorane (controlled flow of 2.5% isofluorane in air will be administered through a nose cone via a gas anesthesia system). Mice are injected i.p. with 150 mg/kg of D-Luciferin Potassium-salt (Goldbio) dissolved in PBS. Mice are imaged 5 to 10 min after injection of luciferin with an IVIS 100 (Xenogen, Alameda, CA) and the data acquisition and analysis are performed with the software LivingImage (Xenogen).

If liver stage luciferase signal is too low for reliable detection, mice are kept until blood stage infection becomes apparent (typically 4-5 days) and imaged again. The intensity of early blood stage infection is proportional to Plasmodium liver load.

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