Leishmaniasis: macrophage in vitro screening model

Parasite and cell cultures

The *Leishmania donovani* strain MHOM/ET/67/L82 (obtained from Dr. S. Croft, London School of Hygiene and Tropical Medicine) is used. The strain is maintained in the Syrian Golden hamster. Amastigotes are collected from the spleen of an infected hamster. Amastigotes are grown in axenic culture at 37°C in SM medium (Cunningham I., J. Protozool. 24, 325-329, 1977) at pH 5.4 supplemented with 10% heat-inactivated fetal bovine serum (FBS) under an atmosphere of 5% CO$_2$ in air. Primary peritoneal macrophages from NMRI mice are collected one day after stimulation of macrophage production with an intraperitoneal injection of 2 ml of a 2% potato starch suspension (FLUKA, Switzerland). All cultures and assays are done at 37°C under an atmosphere of 5% CO$_2$ in air.

Drug sensitivity assays

Stock drug solutions are prepared in 100% dimethylsulphoxide (DMSO) (unless otherwise suggested by the supplier) at 10 mg/ml, and heated or sonicated if necessary to dissolve the sample. After use the stocks are kept at –20°C. For the assays, the compound is further diluted to the appropriate concentration using complete medium. The DMSO concentration in the wells with the highest drug concentration does not exceed 1%.

Assays are performed in sterile 16-well chamber slides (LabTek, Nalgene/Nunc Int.). To each well is added 100 µl of a murine macrophage suspension (4 x 10$^5$/ml) in RPMI 1640 medium containing bicarbonate and N-2-hydroxyethylpiperazine-N’-2-ethane-sulphonic acid (HEPES) and supplemented with 10% heat inactivated FBS (RPMI/FBS). After 24 hrs, 100 µl of a suspension containing amastigotes (1.2 x 10$^6$/ml) are added to each well, giving a 3:1 ratio of amastigotes/macrophages. The amastigotes are harvested from an axenic amastigote culture and suspended in RPMI/FBS. 24 hrs later, the medium containing free amastigotes is removed, the cells are washed once with medium, and fresh medium containing drug dilutions (four 3-fold dilutions for each compound) is added. In this way, four compounds can be tested on one 16-well tissue culture slide. Untreated wells serve as controls. Parasite growth in the presence of the drug is compared to control wells. After 4 days of incubation the culture medium is removed and the slides are fixed with methanol for 10 min and then stained with a 10% Giemsa solution. Infected and non-infected macrophages are counted in the control cultures and those exposed to the serial drug dilutions. The infection rates are determined. The results are expressed as percent reduction in parasite burden compared to control wells, and the IC$_{50}$ is calculated by linear regression analysis (EXCEL Microsoft).

Primary screen

The compounds are tested in duplicate at four concentrations ranging from 9 to 0.3 µg/ml. If the IC$_{50}$ is below 0.3 µg/ml, the range is changed to 1 to 0.03 µg/ml.

- If the IC$_{50}$ is higher than 10 µg/ml, the compound is classified as inactive.
- If the IC$_{50}$ is between 2 and 10 µg/ml, the compound is classified as moderately active.
- If the IC$_{50}$ is < 2 µg/ml, the compound is classified as active and is further evaluated in an in vivo screen.

The standard drug is miltefosine, run in the same assay; the IC$_{50}$ value for miltefosine is 0.33 µg/ml (range 0.19 - 0.49 µg/ml; n = 7).