Minimum inhibitory concentration of drugs against *Mycobacterium leprae* as determined by an *in vitro* assay

R.JAGANNATHAN and P.R.MAHADEVAN
The Foundation for Medical Research, 84-A, R. G. Thadani Marg, Worli, Bombay 400018, India

MS received 3 September 1985

**Abstract.** The observations that live *Mycobacterium leprae* after entry into cultured peritoneal macrophages from mice, reduced the EA rosetting macrophages, have been exploited to determine the minimum inhibitory concentration of diamino diphenyl sulphone and rifampicin. Diamino diphenyl sulphone showed a minimum inhibitory concentration of 0·028 µg/ml and rifampicin 0·11 µg/ml when given externally. However, there was accumulation of diamino diphenyl sulphone inside the macrophages. At an external concentration of 0·028 µg/ml the concentration inside the macrophage was 0·5 µg/ml. The minimum inhibitory concentration for diamino diphenyl sulphone in this assay system is higher by several folds and that for rifampicin is slightly lower, than what is reported earlier with mice foot pad experiments. The minimum inhibitory concentration reported in this assay system is quite close to what is observed for *in vitro* inhibition of *Mycobacterium leprae* with both the drugs.

**Keywords.** *Mycobacterium leprae*; minimum inhibition concentration; drugs; *Fc* receptor assay.

**Introduction**

Among the drugs that are in clinical use against leprosy the diamino diphenyl sulphone (DDS) and rifampicin are most widely used, either individually or as components of recently introduced multidrug therapy (WHO, 1982).

Due to nonavailability of an *in vitro* drug assay using *Mycobacterium leprae*, the minimum inhibitory concentration (MIC) has been reported using experimentally infected mice. The MIC for DDS has been determined as 0·003 µg/ml (Peters et al., 1975) and for rifampicin as 0·3 µg/ml (Holmes and Hilson, 1972) based on the serum level. While this is an interesting and useful piece of information, less confidence is placed on these values in determining the dose of the drug given to the patients. The conventionally administered dose of 100 mg/day and 600 mg at a time, for DDS and rifampicin respectively, according to Allard (1980), provides a peak serum level of 500 and 30 times the MIC. This is given perhaps, with the idea of avoiding development of drug resistant *M. leprae* in the patients. Thus the heavy dose of drug has no relationship to the MIC as determined in mice. It is thus useful to have a much more direct *in vitro* method of determining susceptibility of *M. leprae* to these drugs and the MIC.

Abbreviations used: DDS, Diamino diphenyl sulphone; MIC, minimum inhibitory concentration; SRBC, sheep red blood cells.
It has been demonstrated that the presence of live *M. leprae* inside the macrophages from human or mice, reduced the level of $F_c$ receptor expressing cells. This was not obtained with killed *M. leprae* (Birdi et al., 1983; Birdi and Antia, 1984; Mankar et al., 1984). The basic conclusion derived from this study was that live *M. leprae* but not inactive *M. leprae* were able to alter the surface structure of macrophages. This surface structure alteration resulted in the reduction of $F_c$ receptor expressing macrophages only in the presence of live *M. leprae*. Thus if drugs are active on *M. leprae*, then in the presence of the drug and *M. leprae*, $F_c$ receptor expressing macrophages will not be reduced and the level will be as good as the control (Birdi and Antia, 1984; Mankar et al., 1984).

In this report we present data indicating the MIC of DDS and rifampicin that would inactivate *M. leprae*, such an inactivation being determined using the above *in vitro* test system.

**Materials and methods**

**DDS uptake by macrophages**

Macrophages from the peritoneal cavity of Swiss white mice were obtained and cultured in Leighton tubes as described previously (Mankar et al., 1984). Seventy two hours after distribution of macrophages, the drug DDS was added to the medium. After 3 days of exposure to the drug, the cells were harvested by scraping them with a rubber policeman. They were washed 3 times with saline (0·85 %) to remove extracellular DDS. After suspending the macrophages in 1 ml saline, the cell count was taken in a haemocytometer. The macrophages were then lysed by subjecting them to 8 cycles of freeze-thawing. Distilled ethyl acetate (AR grade) was added to extract DDS from the macrophages. The extraction was done thrice by adding 2 ml ethyl acetate each time. To keep the pH basic, 0·5 ml of 10 $\text{N}$ NaOH was added during the first extraction. The mixture was vortexed each time to facilitate the extraction of DDS from the lysate. After separation of the solvent and aqueous phase in 20 min, the ethyl acetate phase was transferred to another tube with a pasteur pipette. To the ethyl acetate phase 0·5 gm of NaCl (AR grade) was added to remove water molecules carried during the extraction. The ethyl acetate phase was then allowed to separate from the NaCl which sedimented at the bottom. The ethyl acetate phase was removed and checked for fluorescence on a spectrofluorimeter (SFM23-Kontron). This assay method provided a sensitivity upto 2 ng/ml for DDS at wavelength settings of 285 nm for excitation and 350 nm for emission. A culture of macrophage, which had not been exposed to the drug otherwise treated exactly the same as the experimental served as control.

DDS (in ng)/10$^6$ macrophages is equal to a fluorescence level of 1 × 10$^6$ experimental macrophages, from which the fluorescence level of 1 × 10$^6$ control macrophages was subtracted. The quantitation was done using a standard curve (experimentally determined) of fluorescence units against increasing concentrations of DDS added to macrophage cultures.