Antimicrobial activities of dihydrofolate reductase inhibitors, used singly or in combination with dapsone, against *Mycobacterium ulcerans*

Arvind M. Dhople*

*Department of Biological Sciences, Florida Institute of Technology, Melbourne, FL 32901, USA*

Development of new treatments against *Mycobacterium ulcerans* infection has become crucial because of its wide-scale prevalence throughout the world. The effects of dihydrofolate reductase inhibitors, used either singly or in combination with dapsone against *M. ulcerans* were evaluated *in vitro*. When used singly, epiroprim was the most potent, with MICs between 0.5 and 1.0 mg/L, while trimethoprim was totally ineffective. The MICs of K-130 and brodimoprim ranged from 1.0–2.0 mg/L for the former to 2.0–16.0 mg/L for the latter. When combined with dapsone, synergic effects were observed with epiroprim. These results indicate the great potential of epiroprim in treating *M. ulcerans* infections.

**Introduction**

Infection with *Mycobacterium ulcerans* causes indolent, necrotizing ulcers of the skin in man, and clusters near rivers and swamps of tropical or subtropical regions. Even though these ulcers were first described in Uganda in 1897, the causative agent was isolated in Australia in 1948. Thus, the infection is called ‘Bairnsdale ulcer’ after the main town in the original endemic region in southern Australia, and ‘Buruli ulcer’ after a region in Uganda associated with a large number of cases during the late 1960s and early 1970s. The lesions often start as small subcutaneous nodules, which gradually enlarge over days to weeks and lead to painless and chronic ulcers with characteristic undermined edges. Even after more than 50 years, the incidence of infection with *M. ulcerans* seems to be increasing sharply; in the past decade, incidence of this disease has increased dramatically, with cases now reported in most of sub-Saharan Africa, Mexico, Surinam, Peru, Bolivia, French Guiana, India, sporadically throughout southern Asia and in Papua New Guinea. Therefore, the World Health Organization has recently declared Buruli ulcer as an emerging public health problem.

Despite promising results *in vitro* and in laboratory animals, the treatment of these ulcers has been disappointing. Surgery is widely regarded as the definitive treatment, removing necrotic tissue. Early pre-ulcerative lesions can be treated effectively with rifampicin alone or by heating at 40°C. However, rifampicin is not usually effective against advanced ulcerative lesions.

*Corresponding author. Tel: +1-321-674-7253; Fax: +1-321-674-7238; E-mail: adhople@fit.edu*
4-benzyl-pyrimidine] was obtained from Professor Seydel, Forschungszentrum Borstel (Borstel, Germany); trimethoprim [2,4-diamino-5-(3,4,5-trimethoxybenzyl) pyrimidine] and dapsone [4,4′-diaminodiphenyl sulphone] were obtained commercially from Sigma Chemical Co. (St Louis, MO, USA). Stock solutions were first prepared by dissolving the individual compounds in small quantities of dimethylsulphoxide and then diluting further with distilled water. Each working solution was then filter sterilized through a GA-6 membrane filter (pore size 0.22 μm; Gelman Sciences, Ann Arbor, MI, USA).

The procedures described by Heifets and co-workers were followed to determine minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), and also to assess the combined inhibitory effects.

For each sample, control as well as with drug, triplicate assays were performed in each case, and statistical significance was determined by Student’s t-test.

Results

The effects of four dihydrofolate reductase inhibitors, along with dapsone, incorporated singly into 7H12 medium, against M. ulcerans are presented in Table I. The effect of an individual drug was evaluated by determining the inhibition in growth of M. ulcerans caused by that drug as compared with the growth of M. ulcerans in control cultures without any drug. The results presented in Table I are highly significant, with \( P \) values \( \leq 0.05 \). Among the dihydrofolate reductase inhibitors, epiroprim was the most potent in killing M. ulcerans. The MIC for both the type strains was 0.5 mg/L; among the clinical isolates, the MIC for four strains was 0.5 mg/L whereas the growth of the other two isolates was inhibited at a concentration of 1.0 mg/L. More importantly, with epiroprim, the MBC/MIC ratio was 1.0 for seven out of eight strains. The MICs for brodimoprim and K-130 ranged from 2.0 to 16.0 mg/L and 1.0 to 2.0 mg/L, respectively. Trimethoprim was totally ineffective even at a concentration of 16 mg/L. In the case of both brodimoprim and K-130, the values for the MBC/MIC ratio ranged between 2 and 4. Similarly, the MIC of dapsone against all the strains of M. ulcerans ranged between 0.5 and 4.0 mg/L.

When each of the four dihydrofolate reductase inhibitors was combined with dapsone, with each drug at a concentration that was lower than its MIC, very interesting results were obtained and they are presented in Table II. Again, epiroprim was proved to be the most potent drug, exhibiting strong synergy when combined with dapsone. In six out of eight strains, the FIC was 0.5 or below, while in two strains it was 0.75. The combination of K-130 and dapsone exhibited synergy against only two strains, while the effects were additive for the remaining six strains. On the other hand, only additive effects were observed for all eight strains with the combination of brodimoprim and dapsone.
Since trimethoprim was ineffective in inhibiting the growth of *M. ulcerans*, its effects in combination with dapsone were not evaluated.

**Discussion**

Inhibitors of folate biosynthesis are used routinely in the treatment of infectious diseases. Sulphones, such as dapsone, inhibit 7,8-dihydropteroate synthase, whereas 2,4-diamino-5-benzylpyrimidines, such as trimethoprim, selectively inhibit 7,8-dihydrofolate reductase. However, the activity of these benzylpyrimidines is restricted to Gram-negative bacteria. The data presented here clearly show that both dihydrofolate reductase inhibitors and dapsone inhibit the *in vitro* growth of *M. ulcerans*, and epiroprim is more potent than the others. The data also indicate a strong synergic activity between epiroprim and dapsone against *M. ulcerans*. It is interesting to observe that trimethoprim alone is not effective against *M. ulcerans*, though it is widely used against Gram-negative organisms, either singly or in combination with sulphanethoxazole.

We have previously shown excellent synergy between brodimoprim and dapsone against *M. leprae*, both *in vitro* and in mice. Similar findings were also reported recently with the combinations of K-130 and dapsone and of epiroprim and dapsone against *M. leprae*. Thus, it seems appropriate to evaluate further the effects of combining epiroprim and dapsone against *M. ulcerans*. It is interesting to observe that trimethoprim alone is not effective against *M. ulcerans*, though it is widely used against Gram-negative organisms, either singly or in combination with sulphanethoxazole.

At some stage of the disease, most of the patients with *M. ulcerans* infection carry massive bacterial loads and this creates an ideal situation for the selection of drug-resistant mutants. In such a situation, as in any other mycobacterial infection, combined therapy may be advantageous. Thus, the combination of epiroprim and dapsone, along with rifampicin, could have great potential in the clinical treatment of advanced ulcers.

**Acknowledgements**

The author wishes to thank F. Hoffmann-La Roche & Co., Ltd, for the generous supply of epiroprim and brodimoprim, and Professor J. K. Seydel of Forschungszentrum Borstel, for supplying K-130. The author is also grateful to Professor F. Portaels of Institute of Tropical Medicine, Antwerp, Belgium, for supplying clinical isolates of *M. ulcerans*.

**References**


Received 13 July 2000; returned 16 August 2000; revised 7 September 2000; accepted 27 September 2000