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The killing of *Mycobacterium leprae* in mice by various dietary concentrations of clofazimine and ethionamide

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Summary The bactericidal activity for *Mycobacterium leprae* in mice of a range of dietary concentrations of clofazimine and ethionamide was studied by the proportional bactericidal technique. The 2 highest concentrations of clofazimine studied, 0·01% and 0·003% were respectively 99·6 ± 0·2% and 98 ± 1·0% bactericidal. Though less killing was afforded by 0·001% and 0·0001% clofazimine, even the latter concentration retained significant bactericidal activity (84 ± 10% bactericidal). The minimal bactericidal dietary concentration of ethionamide was found to be approximately 0·02% (68 ± 13% bactericidal). Higher concentrations of ethionamide, 0·05% and greater, were consistently more active, at least 95 ± 3% bactericidal. It is noteworthy in these studies that significant bactericidal activity of both clofazimine and ethionamide was retained at lower dietary concentrations than had been demonstrated previously.

Introduction

Actual bactericidal activity against *Mycobacterium leprae* appears critical in the therapy of lepromatous leprosy. Recently results[1] were presented quantifying the killing of *M. leprae* in mice by a wide range of dietary concentrations of dapsone and rifampicin. Previous work[2] in this regard had evaluated only high dietary concentrations of these and other agents which yield steady state levels corresponding to peak levels experienced by patients undergoing therapy. The evaluation of the killing of *M. leprae* by lower dietary concentrations that result in levels that are also experienced by patients had not previously been evaluated by these same methods. Since clofazimine and ethionamide are the only other antimicrobial agents that are widely used to treat leprosy,[3] we studied these 2 antimicrobial agents in a similar manner.

Both clofazimine and ethionamide have been demonstrated to be bactericidal for *M. leprae*-infected mice at high dietary levels. Clofazimine 0·003% and 0·01% in mouse food was previously found to be respectively 96–99% and 98% bactericidal,[2] while ethionamide 0·1% and 0·2% was found respectively 98·6% and 97·4% bactericidal.[2] Clofazimine accumulates in skin, resulting in a dose-related red-black discolouration which makes the drug unacceptable to some patients. If the dosage of clofazimine could be reduced without loss of significant antimicrobial potency, this would certainly be advantageous. Ethionamide is not well tolerated in a significant number of patients because of dose-dependent gastrointestinal intolerance, is hepatoxic, especially when combined

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with rifampicin,[4] and is costly. Therefore ethionamide dosage reduction, if associated with retained antimicrobial activity, would be advantageous. Thus these studies were initiated.

Methods and procedures

The killing of a single strain of *M. leprae* by various dietary concentrations of both clofazimine and ethionamide was quantitated by the proportional bactericidal test.[2] The strain utilized was originally obtained from a patient, long maintained in mice and different from the one utilized in our previously reported studies on the killing of *M. leprae* by various dietary concentrations of dapsone and rifampin.[1] In these studies groups of 10 BALB/c female weanling mice were inoculated with 10, 100, 1000, and 10,000 *M. leprae* in both hind footpads. Clofazimine (Ciba Geigy) and ethionamide (Sigma) diets were prepared from 95% ethanol solutions and evenly distributed in mouse food utilizing a Patterson Kelly twin-drum diet-mixing machine. Study mice were fed a control diet of various dietary concentrations of clofazimine or ethionamide for the first 60 days. Clofazimine was administered to mice in dietary concentrations of 0·0001%, 0·001%, 0·003%, and 0·01%, and ethionamide in concentrations of 0·02%, 0·05%, 0·16%, and 0·3%. One year after completion of therapy, footpads, generally from 5 mice (10 feet), were harvested individually, and *M. leprae* enumerated from each. If the number of *M. leprae* detected were $\geq 5 \times 10^4$, live bacilli were judged to have survived therapy. From these results, the percentage bactericide and the standard error were calculated by the method of Spearman and Karber.[5]

Results

The results of the killing of *M. leprae* by clofazimine are presented in Table 1. Over the range of dietary concentrations of 0·0001% to 0·01% clofazimine resulted consistently in significant bactericidal activity. Clofazimine, 0·01%, was impressively bactericidal, 99·6% ± 2%, and produced more killing of *M. leprae* than the 3 lower dietary concentrations of clofazimine studied ($p < 0.01$). Clofazimine 0·003% produced 98 ± 1% killing, and resulted in more bactericidal activity ($p < 0.01$) than both 0·001% and 0·0001%. The killing of *M. leprae* by 0·001% and 0·0001% clofazimine (94 ± 4% and 84 ± 10% respectively) were indistinguishable ($p = 0.13$).

The results of the killing of *M. leprae* by ethionamide are presented in Table 2. Ethionamide, 0·05%, 0·16%, and 0·3% in diet, produced equivalent killing ($p > 0.05$) of *M. leprae*, respectively

Table 1. The killing of *M. leprae* by clofazimine

Treatment	Inoculum size (*M. leprae* per footpad) 10^4	10^3	10^2	10^1	Proportion of viable *M. leprae**	*M. leprae* killed +S.E.
Control	9/10†	9/10	10/10	8/10	0·0869	—
Clofazimine 0·0001%		10/10	6/10	2/10	0·0138	84·1 ± 10%
Clofazimine 0·001%		10/10	4/10	0/8	0·0055	93·7 ± 4%
Clofazimine 0·003%	8/8	8/10	0/10	0/10	0·0014	98·4 ± 1%
Clofazimine 0·01%	4/4	1/8	1/10	0/6	0·00037	99·6 ± 0·2%

* Derived from $0.69/ID_{50}$.

† Number of footpads showing multiplication of *M. leprae*/number of footpads harvested.

Table 2. The killing of *M. leprae* by ethionamide

Treatment	Inoculum size (*M. leprae* per footpad) 10^4	10^3	10^2	10^1	Proportion of viable *M. leprae**	*M. leprae* killed +S.E.
Control	8/8†	10/10	10/10	6/10	0·0869	—
Ethionamide 0·02%	10/10	10/10	9/10	2/10	0·0275	68 ± 13%
Ethionamide 0·05%	10/10	7/10	0/10	1/10	0·0014	98 ± 1%
Ethionamide 0·16%		9/10	10/10	1/10	0·0022	97 ± 1%
Ethionamide 0·3%	8/8	10/10	2/6	0/6	0·0047	95 ± 3%

* Derived from $0{\cdot}69/ID_{50}$.

† Number of footpads showing multiplication of *M. leprae*/number of footpads harvested

98% ± 1%, 97% ± 1%, and 95% ± 3%. Ethionamide, 0·02%, also resulted in a minimal but significant ($p = 0{\cdot}03$) bactericidal effect, 68 ± 17%; its bactericidal activity was, however, decidedly less ($p < 0{\cdot}01$) than each of the 3 higher studied concentrations of ethionamide.

Discussion

Shepard established that on continuous administration the minimal effective dose of clofazimine in mice was 0·0001%.[6] In our study the activity of clofazimine for *M. leprae* was found to be bactericidal, thus confirming previous studies in mice of Levy[7] and Colston.[2] Levy[6] studied in *M. leprae*-infected mice the effect of various dietary concentrations of clofazimine by the kinetic technique,[8] wherein treatment is administered during a limited period during the logarithmic multiplication of *M. leprae*. By this method, a delay in multiplication of *M. leprae* for a period of time longer than the period of drug administration which cannot be explained by drug accumulation is considered to represent bactericide.[8] Levy[7] found 0·0001% clofazimine to be inhibitory against a strain of *M. leprae* but not bactericidal, while 0·001% and 0·01% clofazimine were found to exhibit bactericidal-type activity. Though clofazimine accumulates in the tissues of mice and is excreted slowly, the delay in the resumption of multiplication of *M. leprae* in mice treated with 0·001% and 0·01% clofazimine in this study was felt to be more than could be accounted for by drug accumulation alone. Colston[2] found, utilizing as we did the proportional bactericidal technique, 0·003% and 0·01% clofazimine to be 96–99% bactericidal. Our results of 0·003% and 0·01% are remarkably similar to those of Colston, 98% and 99·6% bactericidal respectively. In our studies 0·001% and even 0·0001% clofazimine, though resulting in less killing than higher dietary concentrations, retained significant bactericidal activity, 94% and 84% respectively.

For clofazimine it is particularly hazardous to extrapolate from studies in mice to man because of vastly different half lives in the 2 species, 1 week in mice and 70 days in man, and complexities of drug absorption, metabolism and tissue accumulation that remain largely unknown.[7] Nonetheless, these studies demonstrate that bactericidal activity of clofazimine for the studied strain is retained at even very low dietary concentrations of clofazimine. If the bactericidal activity of low dietary concentrations of clofazimine found for this strain is established for the majority of strains of *M. leprae*, dosage reduction of clofazimine in man from the customary 50 to 100 mg daily could be considered.

The earliest studies by Shepard[9-11] on the activity of ethionamide against *M. leprae* in mice utilizing the kinetic technique found 0·001% inactive, 0·01% bacteriostatic, and 0·1% or 0·2%

bactericidal. Colston[12] established in mice that against 3 strains of *M. leprae* continuous feeding of 0·01% ethionamide was consistently effective and 0·003% ethionamide was partially effective. More recently, utilizing continuous feeding of ethionamide in mice, Shepard *et al.*[13] found 0·01% inhibited the growth of *M. leprae*, while 0·003% did not. Thus previous studies have consistently demonstrated that the minimal inhibitory dietary concentration of ethionamide in mice is in the range of 0·003–0·01%. On the other hand, by the kinetic technique Colston[12] found 0·03% to have no demonstrable bactericidal activity, which both 0·1 and 0·2% did. Thus previously both Shepard and Colston independently demonstrated at least a 10-fold discrepancy between the minimal inhibitory and minimal bactericidal dietary concentration of ethionamide for *M. leprae* in mice.

We found that the bactericidal activity against *M. leprae* by the 2 highest studied dietary concentrations of ethionamide, 0·15 and 0·3%, is 95 and 97% respectively; our results are thus in accord with those of Colston[2] using similar methods and concentrations. It is noteworthy, however, that in our studies 0·05% ethionamide was equally bactericidal (98%). The bactericidal activity of 0·05% ethionamide had not been assessed by Shepard or Colston. Though very significantly less bactericidal (68%) for *M. leprae* in our studies, even 0·02% ethionamide proved to have a significant lethal effect on *M. leprae*. We have thus found for the strain of *M. leprae* studied that the minimal bactericidal dietary concentration in mice of ethionamide is about 0·02%. This is several fold less than that established by other investigators.

The establishment of a lower minimal bactericidal concentration for ethionamide has potentially important clinical implications. It is noteworthy that there is a linear relationship between mouse dietary concentrations of ethionamide and resultant serum levels, 0·02% in diet yielding mouse serum levels of 0·1 μg/ml and 0·05% in diet yielding mouse serum levels of 0·2 μg/ml.[12] In man 500 mg ethionamide results in peak serum levels of 3 μg/ml,[14 16] which is more than 10-fold greater than the minimal bactericidal serum concentration established for the studied strain, and has a half-life of 2 hr. Owing to gastrointestinal toxicity in man, ethionamide 1 g/day is poorly tolerated, 500 mg/day better tolerated, and 250 mg/day well tolerated,[17,18] Thus the therapeutic index for ethionamide is quite narrow, and if the relatively low levels of ethionamide required for killing the studied strain can be substantiated as representative, ethionamide doses of 250 mg/day or even less might be sufficient to treat leprosy.

In any case, a clinical trial in the Philippines that is now in progress utilizing daily doses of 250–500 mg ethionamide or prothionamide should determine whether such dosage reduction is effective or not. In future clinical trials, it would be of considerable interest to establish minimal bactericidal concentrations of individual *M. leprae* strains to the agents being utilized in order to assess whether any variations found therein are important in determining the rate of loss of viable *M. leprae* from the skin.

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NEWS AND NOTES

XIIIth International Leprosy Congress, 11–17 September, 1988, The Hague, The Netherlands

Full details of the sessions have already been printed in this journal; see Number 1, **58,** 1986.

The Congress Location and Hotel Accommodation: The 13th ILA Congress will be held in The Netherlands Congress Centre, The Hague, The Netherlands, from 11–17 September 1988. Hotel accommodation will be provided in several price categories ranging from ca. Dfl. 50,-to Dfl. 250,- and more. *Congress Bureau*: For all information concerning the congress, please contact the Congress Bureau: QLT Convention Services, Keizersgracht 792, 1017 EC Amsterdam, The Netherlands. Tel. +31 (0)20-26 1372, Tlx. 31578 inter nl att qlt. This Meeting is co-sponsored by the World Health Organization.

XIIth International Congress for Tropical Medicine and Malaria, September 1988

This congress will be held in the International Congress Center RAI in Amsterdam from 18–24 September 1988, immediately after the International Leprosy Congress (above).

Information can be obtained at: Organisatie Bureau, Amsterdam, Europaplein 12, 1078 GZ Amsterdam, The Netherlands. Tel. +31 (0)20-440807, Tlx. 13499.

Meeting of the International Society of Dermatology, Oxford, September 1988

A joint meeting of this Society with the International Society of Dermatopathology will take place in Oxford, UK, 4–8 September, 1988. There will be at least two sessions on leprosy, including histopathology, together with exhibits and demonstrations, one of which will come from the Wellcome Institute of Tropical Medicine in London. Further details; Mrs Christine Cherry, Department of Dermatology, the Slade Hospital, Headington, Oxford, OX3 7JH, England.

Field Laboratory Services for Leprosy, Calcutta, March 1987

Dr D S Chaudhury, Project Director, Greater Calcutta Leprosy Treatment and Health Education Scheme, has kindly provided the following account of this workshop.

The Workshop was held on 28 February and 1 March. The papers that were presented in the Scientific Sessions covered broadly the following items:

Standardization of smear techniques, grading of bacteriological index, morphological index and its importance, importance of cross-checking of smears, smear workload with reference to population and caseload, smear taking in the field by a Paramedical Worker vis-a-vis a Smear Technician and training needs with particular reference to standardization of training in learning field laboratory work.

2 The Second Scientific Session dealt with field testing of drug compliance, role of histological examinations in National Leprosy Eradication Programme, animal models, procurement and supply of stores and equipments and laboratory forms—some suggestions for the improvement etc.

The Scientific Sessions were followed by open-house discussions which covered topics, e.g. minimum essential requirements for field laboratory services under NLEP, field investigations with particular reference to Multidrug Therapy Districts, up-grading of laboratory facilities in the Regional Training Centres and assessment of requirements of man-power and equipment to maintain optimum quality of work.

The proceedings revealed a number of deficiencies in the existing services for skin smears in leprosy. Remedial measures were discussed in detail. Source: Dr D S Chaudhury, as above, 35/1/A Old Ballygunge, 1st Lane, Calcutta 700 019, India.

Aids for Living: disability prevention

This is a new publication on low-cost technologies for the prevention of disability and the rehabilitation of disabled people throughout the developing world. Free of cost to those working in developing countries. Apply AHRTAG, 85 Marylebone High Street, London W1M 3DE, England.

International Disability, Education and Awareness (IDEA)

This organization continues to keep us informed of courses which are run in the UK on disability and rehabilitation in the developing world; intended primarily for rehabilitation workers and those with a background in occupational/speech therapy, orthopaedics, nursing, social work, psychology, special education. Apply: Director, William House, 101 Eden Vale Road, Westbury, Wiltshire BA13 3QF, England.

Victoria Hospital, Dichpalli, India

We were glad to receive the Annual Report for 1986. Multiple drug therapy has been used with considerable success and the total number of registered patients is coming down. MDT was introduced in late 1982 and by the end of 1986, 453 patients had completed treatment and remain on review. By the end of 1986, there were in fact only 101 patients on the treatment register. Only a negligible number of patients have developed reactions while on MDT. Victoria Hospital, Dichpalli-503 175, Nizamabad District, Andhra Pradesh, South India.

Social Work Techniques for Paramedical Workers, India

We have received a copy of this excellent booklet, produced by the German Leprosy Relief Association and Leprosy Relief Work Emmaus-Switzerland, prepared by the Consultative Committee in India (COCO) and published in India. The contents begin with 'Why do patients not come for treatment?' and end with 'How can you communicate effectively?' Apply: Regional Secretariat for India GLRA-ALES, 4 Gajapthy Street, Shenoy Nagar, Madras 600 030, India.

The use of slides in teaching, ASME

This Medical Education Booklet (Number 13) comes from the Association for the Study of Medical Education, 150b Perth Road, Dundee DD1 4EA, Scotland and is written as a practical guide to the use of slides in teaching. Although only 16 pages in length it is superb and should be compulsory reading for everyone, including doctors and scientists, who present slides at lectures and congresses (and might, with enormous benefit to the audiences, form the basis of required procedues for the forthcoming Leprosy Congress in the Hague, 1988, see page 411).