

Reply to Dr. Levy

TO THE EDITOR:

Dr. Levy has raised some questions about our findings on pyrazinamide reported in the INTERNATIONAL JOURNAL OF LEPROSY recently (⁶). We have been fully aware of

various issues raised by Dr. Levy, and have considered these in depth even before reporting our results. In the following paragraphs we would like to clarify these questions: a) criteria of multiplication, b) reasons

for using drugs which do not show desired activity in the mouse foot pad, and c) our conclusions.

Dr. Levy has commented that we have not mentioned the criteria of multiplication in the mouse foot pad. We have given detailed methodologies of the mouse foot pad technique used by us in our paper (last paragraph of Patients and Methods, page 2). A 10-fold increase in the harvest count compared to the inoculum was taken as true multiplication in this study⁽⁶⁾. This is clearly mentioned in the last line of the above paragraph.

The issue of injunction against the use of drugs which do not show any activity in the mouse foot pad should be dispassionately reexamined. We agree with Dr. Levy that patients should be protected from unnecessary hazardous trials. But we do not accept that the drugs should be discarded just on the basis of mouse foot pad results alone. We have partly discussed this issue in the Introduction and Discussion of our paper⁽⁶⁾. The reasons for our using such drugs are:

a) Because of the uncultivability of *Mycobacterium leprae* in *in vitro* systems, the mouse foot pad model is the only accepted system for drug sensitivity screening. However, all results in mice cannot be extrapolated to humans. For example, clofazimine (Lamprene) (B663) had a very good effect on *M. tuberculosis* infection in mice^(5,12) but clearly was not so effective in human pulmonary tuberculosis, thus results in humans did not correlate with the results in mice. Even in the mouse foot pad model different sensitivity results for *M. leprae* have been reported for thiosemicarbazones^(7,11). Despite the reported 99.9% kills in mice with a single dose of rifampin, viable bacilli are present even after 24 months of multiple drug therapy (MDT) in patients. This is additional evidence that the mouse foot pad is not perhaps the model for the phenomenon of "persistence" or for the testing of drugs for this purpose.

b) Pyrazinamide has a very good sterilizing effect against tuberculosis when it is used as part of a combination therapy in the initial phase of treatment. Pyrazinamide has been reported to inhibit tubercle bacilli directly and/or through its metabolites and acts in the acidic environment of macro-

phages⁽¹³⁾. Since *M. leprae* resides mainly in macrophages, it would be of interest to see its effect in human leprosy. Although it is true that *M. leprae* growth was not inhibited by pyrazinamide in mice when this drug was administered alone⁽¹⁰⁾, this is not definitive evidence to show that it will not have any effect on the prevention of emergence or eradication of persisters in humans, particularly when it is tried as part of MDT. Like clofazimine, the therapeutic effects of pyrazinamide in human cases may be different from the effects in the mouse model. This may be due to pharmacokinetic or metabolic differences, the unsuitability of mice for studying the persister stage, or some unknown factors. This can only be determined by actual trials. With all these background data and knowledge that all studies in animals cannot always be extrapolated to man, we have tried using a combination of drugs with pyrazinamide to see its effect on persisters. Pyrazinamide is an easily available and marketed drug for use against tuberculosis. The trial was well supervised, so we do not believe that patients were in any way put to unnecessary risk.

c) About the use of isoniazid (INH) and thiacetazone, we again felt justified in the same way as for pyrazinamide. Besides that, thiacetazone had been shown earlier to be effective in mice⁽²⁾ and also in man⁽³⁾.

The combination of INH and thiacetazone has been in use against tuberculosis for several years and, despite occasional toxicity, is well tolerated. Shepard⁽⁹⁾ has also reported the bacteriostatic effect of INH in mice when administered continuously, but not by the kinetic method. Beneficial effects have also been recorded in clinical trials⁽⁴⁾. Besides the present study, the combination of dapsone (DDS) + INH + thiacetazone also has been tried at this institute earlier⁽⁸⁾. Although the number was small, it has been found to be almost as effective as DDS + clofazimine, and not many adverse reactions have been recorded. Most of the present-day antileprosy drugs were considered initially because of their effects against tuberculosis. We believe that anti-tuberculosis activity should also be given some weight, as well as activity in mouse foot pad infections, in considering new antileprosy drugs even though neither of these

guarantees the efficacy in man. That can only be decided by actual trials. We are not recommending their use but see no harm in investigating them in well-supervised trials. The combination of such drugs will have an additional advantage in countries endemic for both tuberculosis and leprosy.

Finally, about our conclusion, we have not tried to make any claim about the efficacy of pyrazinamide. To the contrary, we have indulged in self-criticism and highlighted various limitations of our work. Since the corresponding regimens with pyrazinamide showed growth in far smaller numbers of patients at 2 years (1/20) as compared to regimens without pyrazinamide (9/38), we inferred that pyrazinamide may have some effect. We have discussed various limitations (Discussion, page 7, second paragraph, second column, and also Summary) of our study in our paper. The number of cases from whom scrotal biopsies could be obtained was small. We believe that the duration of 2 months' treatment was also inadequate, and thymectomized irradiated mice should have been used but were not available at our institute then. Nevertheless, we have done the statistical evaluation by the "Z" test for proportions for mouse foot pad results at 2 years and find the results significant ($p < 0.05$). The level of significance by Fisher's exact test was < 0.07 . The statistical details were not mentioned in the report because we believe that statistical analysis at this stage would not serve any purpose. In this preliminary communication, we wanted to share our experiences and not make any hasty definitive conclusions.

To quote from our paper, the tentative conclusion was that "Pyrazinamide appears to have some effect against persisters in leprosy, and a well-controlled, randomized trial with longer duration of pyrazinamide therapy in a larger group of patients needs to be carried out to unequivocally determine the exact role of pyrazinamide in leprosy." These are the inferences which we have drawn and also mentioned in the concluding sentences of the Discussion and also in the Summary of our paper.

We thank Dr. Levy for his comments and hope that our reply will explain our logic as well as our interpretations.

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