

Studies on Sulfone Resistance in Leprosy

2. Treatment with a Riminophenazine Derivative (B.663)¹

John H. S. Pettit and R. J. W. Rees²

Until recently no direct experimental methods were available for determining the drug sensitivity of *Mycobacterium leprae* and therefore it was not possible to prove that relapses occurring during treatment were due to the emergence of drug-resistant bacilli. The transmission of human leprosy in the mouse foot pad, first described by Shepard (¹⁵), now provides a method of testing the drug sensitivity of *M. leprae*. Because the sulfones, particularly 4-4'diaminodiphenyl sulfone (DDS) are the most widely used drugs for the treatment of leprosy, we particularly applied the foot pad test to the problem of DDS resistance in patients at Sungei Buloh Leprosarium who were suspected of being resistant to such treatment. The methods for detecting cases of sulfone resistance have been described in the preceding paper (¹¹). Altogether nine patients were studied and four were shown to be infected with DDS-resistant leprosy bacilli. The first seven patients were studied concurrently, many months before the other two (¹⁰), and contributed three of the four cases of sulfone resistance (Cases 3, 4 and 6, patients Nos. 9386, 10458 and 10735, respectively, in the preceding paper). Thus we were faced

with three patients who had failed to improve despite a long history of previous sulfone treatment and a six month course of DDS supervised by our own personnel. It was hoped that they would respond to chemotherapy using a different type of drug and therefore we chose to treat them with one of the riminophenazine derivatives, B.663 (¹), which had recently been shown to have antileprosy activity (^{4, 5}) and which chemically was completely unrelated to the sulfones.

In this paper we present briefly a report of the successful treatment with B.663 of the first three proven cases of sulfone resistance in leprosy, together with some indication of the side effects produced by this new type of antileprosy drug.

METHOD OF STUDY

Although there were too few patients to envisage any form of controlled trial in this study, the investigations performed before and during therapy, and the methods of assessing progress, were based on protocols used for detailed drug trials by our Research Unit in Malaysia (^{16, 17}). Prior to the start of treatment a clinical examination was performed, including x-ray examination of the chest and urine and blood examinations. The detection of other ailments would not have precluded treatment of the three patients with B.663 because of their urgent need for chemotherapy, but in fact none of them showed any abnormality other than those connected with leprosy. A detailed study of the leprosy

¹Received for publication April 22, 1966.

²John H. S. Pettit, M.R.C.P., The Research Unit, Sungei Buloh Leprosarium, Selangor, Malaysia and National Institute for Medical Research, Mill Hill, London, N.W.7, England. Present address: U. S. Public Health Service Hospital, Carville, Louisiana, U.S.A. 70721. R. J. W. Rees, F. C. Path., National Institute for Medical Research, Mill Hill, London, N.W.7, England.

condition was also made, which included a careful clinical examination with charting of the lesions and the sensory changes, colored photographs, skin smears from six sites, a record of the bacteriologic (BI) and the morphologic (MI) indices, and biopsies of two lesions.

The detailed study reported here is confined to the first 12 months of treatment with B.663. During this period hemoglobin estimations and white and red cell counts were performed monthly, the urine was examined weekly, and the patients were weighed regularly. Smears for assessing the BI and MI were taken from the same six skin sites every month and a half for the first six months and then at three-monthly intervals. At periods of three, six and 12 months careful clinical examinations were made, including biopsies, colored photographs and charting of the patient's lesions. At the end of each clinical examination, an assessment of the patient's condition since the previous examination was made. The presence or absence of lepra reaction or erythema nodosum leprosum (ENL) was recorded in the manner suggested by Waters (¹⁶). It was intended that clinical examination and assessment of the patients should also be made independently by an external assessor who would be ignorant of the treatment, but this proved to be impracticable at the time.

Treatment. One 100 mgm. capsule of B.663 was given by mouth three times a day for six days per week for a period of one year. Although the study reported here is confined to the first year, treatment with B.663 was continued, but at a reduced dose, first to one capsule twice daily for three months and subsequently one capsule daily.

PROGRESS OF THE PATIENTS

Cae 3.³ (No. 9386) Chinese male, age 58. When this patient was shown to be suffering from a sulfone-resistant leprosy infection the BI was 3.7 and the MI was 35.

³The case numbers in this paper correspond with the case numbers in the preceding paper (THE JOURNAL 34 (1966) 375-390).

At the completion of one year on B.663 the patient had improved clinically for the first time in many years; the clinical assessment throughout the period showed steady improvement from one assessment to another and at no time was there any suggestion of regression. The MI fell within the first six weeks to 3.0, the BI slowly improved, and the biopsy index at the end of a year had fallen to almost half the pre-treatment figure. The white cell count showed no abnormality, the hemoglobin rose from 9.1 to 10.5 gm., and the blood pressure and weight remained unchanged throughout the year.

Within six weeks of the start of treatment with B.663, the small, hard lepromatous nodules, which were scattered liberally over the body, had become noticeably redder than the surrounding skin. The urine was first noticed to be pink after eight weeks and remained discolored throughout the treatment. The skin steadily became a dusky color, but at all times the lesions were slightly redder than the other areas. None of the patient's lesions showed the blue-black pigmentation that has been reported in some lighter-skin patients receiving B.663 (^{12, 19}), and he never suffered from diarrhea.

Cae 4.³ (No. 10458) Chinese male, age 50. This patient had severe nodular lepromatous leprosy and in spite of many years' treatment with sulfone looked clinically as if he had never been treated. When treatment was started with B.663, the BI was 4.3, the MI was 52 and the biopsy index was 2.7. Clinical assessment showed steady improvement of the lesions and the patient was delighted with the result. After six weeks of treatment the MI had fallen to 22 and by the end of three months it was 1. At the completion of one year's treatment the biopsy index (which earlier failed to fall) was 1.05. The hemoglobin fell slightly (from 14.5 and 13.2 gm.), but the white cell count and the blood pressure remained unchanged and the weight increased throughout the year.

Within two weeks of the start of B.663 treatment it was noted that his face was slightly pink and after four weeks there was a definite reddening of the skin and

lesions, but in this case there was no noticeable difference between the two. After three months' treatment the patient was worried about the deepening pigmentation, although at no time did any of the lesions become black and throughout the year there were no bowel symptoms. At the end of the year he was not nearly as red as had been expected; on several occasions the urine was not pink and it seemed probable that he failed to take all the drug he was given.

Cae 6.³ (No. 10735) Chinese male, age 43. This patient on entry to the study had enormous plaques of lepromatous infiltration over the arms and hands and extensive infiltration of many other areas of the skin. At the beginning of treatment his biopsy index was 2.25, the BI was 4.3 and the MI 32. At the completion of one year on B.663 the patient still had large indurated plaques of leprosy, which looked most unpleasant to a new-comer because of their unusual dark blue color, but the lesions were in fact only about half their original size and the patient was thoroughly satisfied with the improvement that had been obtained. By the end of the year the biopsy index, which had fallen steadily, was less than half the original and the MI was 0.5. The BI in this patient did not show very much change throughout the year. The hemoglobin, blood pressure and weight remained normal. At the beginning of treatment the white cell count was 13,800, but it rapidly returned to a more nearly normal level and stayed steadily in the region of 6,000-8,000, with a normal differential count.

The patient's face developed a pink color about one month after the start of treatment, and this became steadily more severe during the first two or three months. All the lesions also showed a darkening as well as reddening of the skin and within six months most of them were slate-gray to dark blue in color. This pigmentation has gradually faded with the reduction in the dose of B.663 during the period of 12-18 months. After eight months the patient complained of some diarrhea. It was believed that this was probably due to the use of B.663, as other cases have also shown such symptoms (^{9, 12, 19}), but in this case

the diarrhea was controlled with magnesium trisilicate mixture and it was not necessary to stop the treatment.

ANALYSIS OF RESULTS

Clinical. All three patients improved steadily throughout the year on treatment with B.663. However, each developed some pigmentary changes affecting both the lesions and the surrounding skin. In spite of these changes all the patients were satisfied with their clinical improvement. At the time of writing, after a further 16 months of treatment with B.663, clinical improvement has continued in the three patients.

Bacteriology. Bacteriologic assessments, based on the MI and BI as an average of smears from six skin sites, were undertaken every month and a half for the first six months and at three-month intervals for the second six months. The changes in the MI throughout this period are shown in Figure 1 and the BI, together with the MI at the beginning and end of treatment are shown in Table 1. There was a significant and satisfactory fall in the MI's of the three patients to a level of 5 or less after three months' treatment. Furthermore the MI's remained low throughout the remaining nine months on treatment. These results were consistent with our previous experience, gained from extensive trials of DDS in leprosy (^{16, 17}), and indicated that B.663 is an active antileprosy drug capable of killing leprosy bacilli (¹⁸). The changes in the BI would not be expected to be particularly dramatic over the course of only one year's treatment, but in fact two of the three patients showed some diminution.

Histology. Improvement based on histologic changes during treatment was assessed from the biopsy index (¹⁴) obtained from two biopsies of skin taken from each patient at the beginning and after three, six and 12 months. The overall assessments of the biopsy indices from the beginning to the completion of 12 months' treatment are shown in Table 1. All three patients showed significant improvement with falls of 47, 53 and 61 per cent respectively. This index has proved to be a reliable and sensitive method for assessing the histologic

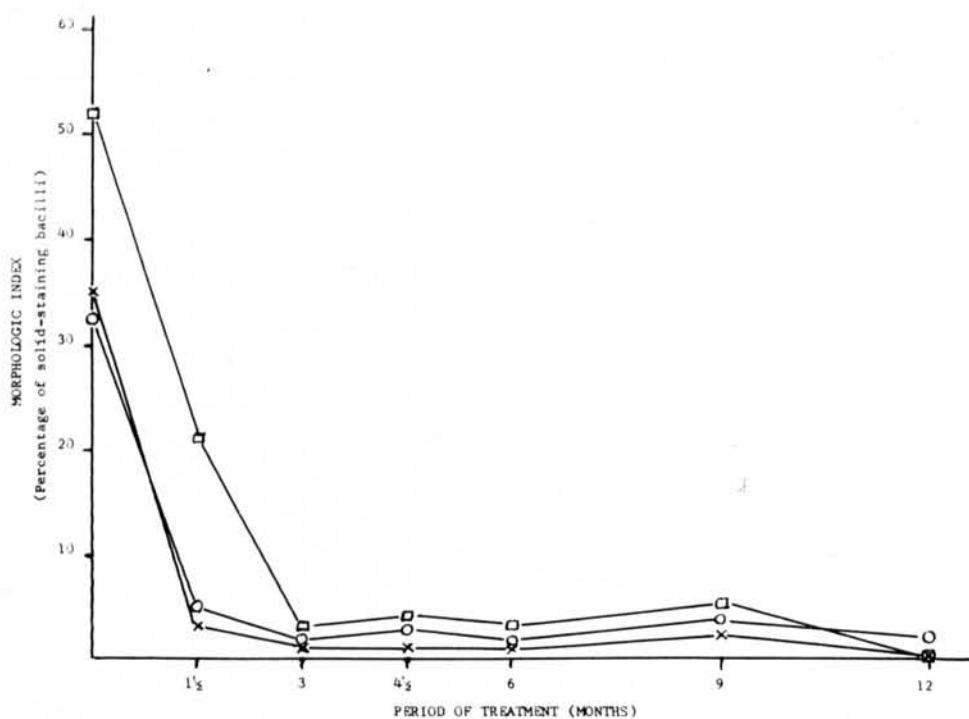


FIG. 1. The effect of treatment with B.663 on the morphologic index (MI) in 3 patients with sulfone-resistant leprosy. X—X Case 3, No. 9386; □—□ Case 4, No. 10458; O—O Case 6, No. 10735.³

progress of a patient to treatment⁽¹⁴⁾ when it is based on a series of skin biopsies from carefully matched and relatively large areas of infiltration. As we have shown⁽¹¹⁾, the method is far less reliable in patients presenting with only small skin nodules, which appears to be a particular feature of patients with sulfone resistance and which

applied to two (Case 3 and Case 4) of the present three patients.

Reactions. Although it was decided at the beginning of the study to observe and assess the development of reactions, either of the lepra or ENL type, by the end of the year's treatment, none of the three cases had shown any reactions at all.

TABLE 1. Bacteriologic and histologic response of the three patients with DDS-resistant leprosy after 12 months' treatment with B.663.^a

Case number	Period of treatment	Morphologic index	Bacteriologic index	Biopsy index
3	Before	35	3.7	2.25
	After one year	0	3.1	1.2
4	Before	52	4.3	2.25
	After one year	0	4.0	1.05
6	Before	32	4.3	2.7
	After one year	0.5	4.5	1.05

^a300 mgm. B.663 daily for six days per week.

Furthermore, at the time of writing, when treatment with B.663 had been maintained for 28 months, there were still no reactions.

General clinical findings. All three patients developed some pigmentary complications. In two of the patients (Case 3 and Case 4) the pigmentation was red, whereas in the most severely affected (Case 6) the red discoloration became darker and eventually developed into blue-black pigmentation of all his lesions. In all three patients the urine became red within a few weeks, but this became less apparent toward the end of the year's treatment and on many occasions the urine of Case 4 was a normal color. It is suspected that all the patients took rather less than the full dose of drug during the later stages of treatment. None of the other investigations showed any abnormalities and therefore, with the exception of coloration produced by B.663 in the urine, skin and leprosy lesions, the drug showed no toxicity.

DISCUSSION

In our studies on sulfone resistance in leprosy the first objective was to determine if such resistance could be demonstrated by testing the sensitivity of *M. leprae* to DDS by use of the foot pad infection in mice. The preceding paper (¹¹) described the methods used for detecting patients with *prima facie* evidence of sulfone resistance based on a lack of clinical and bacteriologic improvement over at least five years. Nine such patients were detected, of whom four failed to respond to a rigorously controlled six months' trial period of DDS under supervision in our own Research Unit, and bacilli from the same four patients were demonstrated in the mouse foot pad infection to be resistant to DDS. The present paper reports the successful response of three of these patients to treatment with a riminophenazine derivative, B.663.

The availability of B.663 was most welcome, because it could be used not only to treat the patients, but also to demonstrate that drug resistance in leprosy is no different from that in other infections. It is well known that such resistance is specific for the therapeutically active moi-

ety of the drug or series of drugs with a similar mode of action, and it was not expected that sulfone-resistant bacilli would resist treatment with drugs that had another mode of action. The choice of alternative drugs for the treatment of these patients was somewhat limited, as all drugs of the sulfone series, including Sulphetrone and the long-acting sulfonamides, were excluded because of the known cross-resistance which occurs within this series against other bacteria. We were not inclined to use thiacetazone (TB1) or thiambutosine (Ciba 1906) because of the reports that a proportion of patients develop resistance in the second or third year of treatment (^{8, 7}). Because of the severity of the leprosy infection in our three patients it was anticipated that treatment would be prolonged, and we preferred to keep thiambutosine in reserve if another anti-leprosy drug was available. The only promising alternative was to use B.663, as it represents an entirely new type of antimycobacterial drug and is therefore incapable of showing cross resistance with the sulfones. Rigorous clinical, bacteriologic and histologic assessments throughout the first 12 months of treatment with B.663 have all shown satisfactory improvement, and at the time of writing it has been maintained for a total period of 28 months. In particular the fall in the morphologic index (MI), which is considered to be the most sensitive method for assessing therapeutic activity of a drug as a measure of its ability to kill leprosy bacilli (^{13, 18}), indicated that B.663 was an active drug and indeed produced a greater rate of fall in the MI than has been our experience with DDS in previously untreated cases of lepromatous leprosy (^{16, 17}). However, it would not be justified, on the basis of these results on only three specially selected patients, to conclude that B.663 is a more active drug than DDS. These results are similar to findings in other bacterial infections which are drug-resistant in that successful treatment was obtained by a chemotherapeutic agent with a different mode of action from the drug to which the organism was resistant.

In addition to the main investigation of

sulfone resistance this study provided further information on the use of B.663 in the treatment of leprosy. The satisfactory response of these three patients confirmed previous claims made for B.663 as an anti-leprosy drug. In the 12 months assessment period, and the 16 month extension of this, B.663 appeared to be as active as DDS. Furthermore, there was no evidence in this period of observation that resistance to B.663 had developed, although Browne and Hogerzeil (⁶) believed they saw resistance to B.663 after only 12 months of treatment. Because of a suggestion that B.663 suppresses or diminishes the severity of ENL (²) our patients were carefully observed throughout and no such reactions have occurred, but the series is too small to draw any conclusion. Except for slight diarrhea in one of the three patients, a manifestation noticed by others using this drug (^{9, 12, 19}), there was no evidence of systemic complications. However, changes in pigmentation of the skin and leprosy lesions that occurred during treatment with B.663 are a matter of considerable concern. The present three patients all developed some red discoloration of their skin and one blue-black pigmentation of the leprosy lesions. Although Browne (³) believed that the "coloration *per se* should not constitute an insuperable cosmetic objection to the use of the drug," he found it necessary to qualify this by adding "especially in patients living among populations showing a considerable range of skin pigmentation." In other words, although the reddish pigmentation was noticed in some of his patients as early as the tenth day of treatment, the changes in color did not unduly upset an African population. In Chinese patients this degree of discoloration constitutes a much more serious cosmetic objection. The present three patients did not object, probably because it was a small price to pay for obvious improvement after so many years of failure on DDS. However, in a pilot trial of B.663 in previously untreated lepromatous leprosy, Chinese patients were deeply dissatisfied with the drug because of the discoloration it produced, and we have found that other patients have refused

the drug (¹²). Doses of 300 mgm. of B.663 daily for six days a week can hardly be called an acceptable therapy for light skinned patients.

SUMMARY

The first three patients with proven DDS-resistant leprosy infections were treated for one year with the riminophenazine derivative B.663 (300 mgm. daily for six days a week). All of them showed satisfactory clinical, bacteriologic and histologic improvement, which at the time of writing has been maintained for a total period of 28 months. The results show that active leprosy resulting from resistance to one drug can still respond satisfactorily to a different type of drug, as is the case with drug resistance in other bacterial infections.

In this limited study B.663 showed no toxicity, but the degree of skin discoloration was disconcerting to Chinese patients.

RESUMEN

Los primeros tres enfermos con infección de lepra resistente a DDS, fueron tratados durante un año con el derivado de riminophenazina B.663 (300 mgm. diarios durante seis días a la semana). Todos mostraron mejoría clínica, bacteriológica e histológica, la que al momento de escribir el presente artículo se ha mantenido por un período total de 28 meses. Los resultados muestran que la actividad de la enfermedad leprosa, consecuencia de la resistencia a una droga, todavía puede responder satisfactoriamente dándole un tipo diferente de medicamento, en la misma forma cuando hay resistencia a la droga en otras enfermedades infecciosas.

En este estudio limitado, el producto B.663 no mostró signos de toxicidad, pero el grado de decoloración de la piel fué desconcertante para los enfermos Chinos.

RÉSUMÉ

Les trois premiers malades atteints d'une infection lépreuse avec résistance confirmée pour la DDS ont été traités durant une année avec le dérivé de la riminophenazine, B.663, à raison de 300 mgm. par jour six jours par semaine. Tous trois ont témoigné d'une amélioration satisfaisante, aux points de vue clinique,

bactériologique et histologique. Au moment où cet article a été écrit, l'amélioration se maintenait pour une durée totale de 28 mois. Ces résultats démontrent qu'une lèpre active provenant d'une résistance à un médicament peut encore répondre favorablement lorsqu'on administre un autre produit de type différent, ainsi qu'il en est dans le cas de résistance médicamenteuse dans d'autres infections bactériennes.

Au cours de cette étude limitée, le B.663 n'a pas témoigné de toxicité, mais le degré de décoloration cutanée était déconcertant à voir chez des malades chinois.

Acknowledgments. The Leprosy Research Unit is jointly administered by the Malaysian Ministry of Health and the British Medical Research Council. We are particularly grateful to Dr. D. S. Ridley, of the Hospital for Tropical Diseases, London, for the histologic assessments. Our thanks are due to the staff and patients of Sungei Buloh Leprosarium who have cooperated fully with this work, and also to Dr. P. D. Fowler of Geigy, Manchester, for the supply of B.663.

REFERENCES

1. BARRY, V. C. and CONALTY, M. L. The antimycobacterial activity of B.663. *Leprosy Rev.* **36** (1965) 3-7.
2. BROWNE, S. G. "B.663" Possible anti-inflammatory action in lepromatous leprosy. *Leprosy Rev.* **36** (1965) 9-11.
3. BROWNE, S. G. Red and black pigmentation developing during treatment of leprosy with "B.663." *Leprosy Rev.* **36** (1965) 17-20.
4. BROWNE, S. G. and HOGERZEIL, L. M. "B.663" in the treatment of leprosy. Preliminary report of a pilot trial. *Leprosy Rev.* **33** (1962) 6-10.
5. BROWNE, S. G. and HOGERZEIL, L. M. "B.663" in the treatment of leprosy. Supplementary report of the pilot trial. *Leprosy Rev.* **33** (1962) 182-184.
6. BROWNE, S. G. and HOGERZEIL, L. M. Apparent resistance of *M. leprae* to "B.663." *Leprosy Rev.* **33** (1962) 185-189.
7. DAVEY, T. F. Some recent chemotherapeutic work in leprosy. *Trans. Roy. Soc. Trop. Med. & Hyg.* **54** (1960) 199-206.
8. LOWE, J. The chemotherapy of leprosy. Late results of treatment with sulphone, and with thiosemicarbazone. *Lancet* **2** (1954) 1065-1068.
9. PETTIT, J. H. S., MARCHETTE, N. J. and REES, R. J. W. *Mycobacterium ulcerans* infection. Clinical and bacteriological study of the first cases recognized in South East Asia. *Brit. J. Dermatol.* **78** (1966) 187-197.
10. PETTIT, J. H. S. and REES, R. J. W. Sulphone resistance in leprosy. An experimental and clinical study. *Lancet* **2** (1964) 673-674.
11. PETTIT, J. H. S., REES, R. J. W., and RIDLEY, D. S. Studies on sulfone resistance in leprosy. (1) Detection of cases. *Internat. J. Leprosy* **34** (1966) 375-390.
12. PETTIT, J. H. S., REES, R. J. W. and RIDLEY, D. S. Chemotherapeutic trials in leprosy. 3. Pilot trial of a riminophenazine derivative, B.663, in lepromatous leprosy. *Internat. J. Leprosy* **35** (1967). *In press.*
13. REES, R. J. W. and VALENTINE, R. C. Application of quantitative electron microscopy to the study of *Mycobacterium lepraemurium* and *M. leprae*. In *Leprosy in Theory and Practice*, R. G. Cochrane and T. F. Davey, Eds. Bristol, John Wright & Sons, Ltd. and Baltimore, Williams and Wilkins Co., 1964, pp. 26-35.
14. RIDLEY, D. S. Therapeutic trials in leprosy using serial biopsies. *Leprosy Rev.* **29** (1958) 45-52.
15. SHEPARD, C. C. The experimental disease that follows the injection of human leprosy bacilli into foot pads of mice. *J. Exper. Med.* **112** (1960) 445-454.
16. WATERS, M. F. R. Chemotherapeutic trials in leprosy. 1. Comparative trial of Macrocydon plus dapsone and dapsone alone in the treatment of lepromatous leprosy. *Leprosy Rev.* **34** (1963) 173-192.
17. WATERS, M. F. R. and PETTIT, J. H. S. Chemotherapeutic trials in leprosy. 2. Comparative trial of dapsone plus ditophal (Etisul) and dapsone alone in the treatment of lepromatous leprosy. *Internat. J. Leprosy* **33** (1965) 282-296.
18. WATERS, M. F. R. and REES, R. J. W. Changes in the morphology of *Mycobacterium leprae* in patients under treatment. *Internat. J. Leprosy* **30** (1962) 266-277.
19. WILLIAMS, T. W., MOTT, P. D., WERTLAKE, P. T., RUBIO, J. B., ADLER, R. C., HILL, G. J., SUAREZ, G. P. and KNIGHT, V. Leprosy research at the National Institutes of Health: Experience with B.663 in the treatment of leprosy. *Internat. J. Leprosy* **33** (1965) 767-775 (Part 2).