Chemotherapeutic Trials in Leprosy

6. Pilot Study of the Riminophenazine Derivative B.663 in Low Dosage (100 mgm. twice weekly) in the Treatment of Lepromatous Leprosy

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The riminophenazine derivative B.663, first synthesized by Barry et al. (1), was shown by Browne and Hogerzeil in 1962, (2-4) to be effective in the treatment of lepromatous leprosy. This finding has been confirmed by a number of centers (5-8, 10, 12, 16). In particular, at the Leprosy Research Unit, Sungai Buloh Leprosarium, considerable experience has been gained in the use of B.663 in the treatment of relapsed lepromatous leprosy due to the development of sulfone-resistant strains of Mycobacterium leprae (9). All such patients, when treated with B.663 in the dosage of 300 mgm. daily for six days each week, have shown satisfactory clinical, bacteriologic and histologic improvement. A pilot study of B.663, given in the same dosage for five months to previously untreated lepromatous patients (11), produced therapeutic results that were comparable to those obtained with standard sulfone therapy. However, this dose of B.663 invariably resulted in marked pigmentation of the skin, more obvious in lighter-skinned patients, many of whom complained strongly of the discoloration. In addition a small number of patients suffered from periodic mild diarrhea. It was therefore considered essential to study the effect of B.663 in much lower dosage, both on the rate of therapeutic response and on the degree of skin pigmentation developed. The dose of 100 mgm. twice weekly was chosen, and eight lepromatous patients were included in the pilot trial.

METHOD OF STUDY

The selection of patients, the investigations performed, and the methods of assessing progress were based on protocols previously developed at the Leprosy Research Unit, Sungai Buloh (11, 12, 16). Eight patients were selected from untreated patients admitted to the Leprosarium. The intake was restricted to those who were fully lepromatous, i.e., patients whose histology showed no features of borderline condition (15), whose morphologic index (M1) was 25 or more, and who gave no history of treatment with antileprosy drugs (Case 2 may have received one injection of dapsone before admission). In addition, random tests for urinary sulfone, using the method of Bratton and Marshall (5), were performed throughout the period of the trial, to ensure that patients were not surreptitiously taking dapsone (DDS) in addition to the prescribed B.663.

As analysis of previous trials (17) has shown that the rate of response to treatment is unaffected by either sex or race; therefore all patients 12 years old and over were considered for admission. By chance, all were males; racially they included Malays, Chinese, and one Gurkha. Before commencing treatment all patients received a full clinical examination, chest X-ray, urine analysis and blood count to exclude significant intercurrent organic disease. Worm infestation was treated before the start of B.663 therapy. One patient found to be suffering from iron deficiency anemia was treated with ferrous sulfate by mouth throughout the period of the trial.

Treatment consisted of B.663 100 mgm. twice weekly for four and a half months. Patients were examined by an independent clinical assessor both before and at the end.
of the duration of treatment. Similarly, two biopsies were made on each patient at 0 and at 45 months for independent histologic assessment. Smears were made before the start of treatment and at one and a half months' intervals throughout the trial, and were scored independently for both the bacillary index (BI) and the MI. In addition an attempt was made to assess the rate of development and the depth of pigmentation resulting from B.663 therapy; however, it proved impracticable to arrange for the investigation to be carried out on a "blind" basis, and, as a compromise, the member of the scientific staff of the Leprosy Research Unit who was not in clinical charge of the trial patients examined each one briefly once a week to note the presence or absence of pigmentation.

CASE HISTORIES

Case 1. (No. 16104) Chinese male, age 23. This patient stated that some seven to eight years before admission he developed numbness and weakness of his right hand. Two years before being admitted to hospital he noticed thickening and redness of one fingers, and subsequently spots and lumps developed all over his body. On examination, there was widespread infiltration of the skin, with numerous lepromatous nodules and the fingers were particularly infiltrated and thickened. On the trunk and limbs proximally were several borderline annular lesions with anesthetic centers, whose edges were not clear cut but were tending to merge with the surrounding lepromatous infiltrate. The right hand showed radial, ulnar and median nerve weakness and associated muscle wasting; encircling the right arm was an ill-defined annular lesion, distal to which the arm was anesthetic. The clinical impression was that this patient had developed borderline (BR) or even borderline-tuberculoid leprosy (BT) eight years earlier, that his resistance had subsequently fallen, and that he had now progressed through borderline lepromatous (BL) to lepromatous leprosy (LL). The lepromin test was negative, the Mitrosa reaction measuring only 2 mm, at three weeks. The admission smears gave an average BI of 5.0 and MI of 26. The histology showed active lepromatous leprosy (LL), the logarithmic biopsy index (LIB) (13) being 5.4. Tuberculin tests of 1 and 20 TU were negative, both at the start and on completion of the trial.

Case 2. (No. 16107) Malay male, age 45. He gave a three year history of numbness over the right forearm and wasting of the muscles of the right hand. Twenty days before admission he developed an ulcer on his right foot. On examination there was gross lepromatous leprosy of his face and limbs although the infiltrate was not quite confluent on his back. There was symmetric mild nerve enlargement save for the right superficial radial nerve, which was considerably thickened. The intrinsic muscles of the right hand were wasted, and marked glove and stocking anesthesia was present. Clinically the patient showed classic lepromatous leprosy, and histologically he was LL. The LIB was 9.6. Admission smears gave an average BI of 5.0 and MI of 27. The lepromin test was completely negative. The tuberculin test to 1 and 20 TU were negative on admission, and on completion of the trial the patient was still negative to 1 TU but positive to 20 TU, reading 13 mm.

Case 3. (No. 16112) Chinese male, age 54. Three years before entering hospital he noticed that his left ankle was numb. Since that time, both ankles swelled intermittent-ly, and for two years there had been slight numbness of his right ankle. Nine months before admission he developed red patches on his face, body, and limbs, which slowly became worse. On examination he was a thin anxious man. There were multiple ophthalmatous flattened papules and small plaques over much of the body, coalescing over the face and upper limbs but more discrete on the trunk and less numerous on the lower limbs. A large flat plaque covered the left ankle and the lower half of the left leg. Appreciation of pinprick was diminished in this plaque and over both feet. Both superficial radial and both lateral popliteal nerves were slightly enlarged. The blood pressure was 150/100 and there was an apical systolic flow murmur, although the heart was not enlarged. Biopsy revealed active lepromatous (LL) leprosy.
and confirmed the presence of a mild lepra reaction (leprosy exacerbation). The LIB was 5.4, the average BI 4.7 and the MI 20. The lepromin reaction measured 4 mm. at three days and 3 mm. at three weeks. The tuberculin test was negative to 1 TU but gave a reading of 14 mm. to 20 TU; after four and one half months' treatment there was an 18 mm. reaction to 1 TU.

Case 4. (No. 1613) Chinese male, age 12. This school boy noticed a red spot on the right side of his back about one and a half years before he was admitted. He then developed red spots on his thighs and legs and subsequently on his arms, face and nose. On examination there was widespread lepromatous infiltration with scattered nodules, present especially on the face, maculopapules and plaques. Faint infiltrated annular lesions were visible on the buttocks. There was slight symmetric nerve enlargement. Appreciation of pin-prick was impaired over both forearms, the left more than the right, though not on the fingers; it was impaired also over both legs and the lower part of the thighs, although sensation was preserved in the region of the insteps. The fingers were slightly swollen, and the lymph nodes were palpable. The istmus of the thyroid was just palpable. The pretreatment biopsies revealed active lepromatous (LL) leprosy, and the LIB was 5.1. The smears gave an average BI of 4.7 and an MI of 31. The lepromin test was negative, giving a reaction of 3 mm. at three days and 0 mm. at three weeks. The tuberculin test was negative to 1 TU but to 20 TU gave a reaction of 11 mm. at the beginning and 20 mm. at the end of the trial.

Case 5. (No. 16121) Malay male, age 23. More than a year before he was admitted to the leprosarium, this patient noticed a red patch on the right side of his chest. Eight or nine months later he developed patches on his body, face and legs. Small nodules were present on both ears, whereas much of the skin of the face, body and limbs was covered with large coalescing lepromatous macules. In the upper half of the body these were mostly hyperpigmented, although some were erythematous. In the bathing-drawers area many were hypopigmented. There was fairly symmetric slight nerve enlargement. The right third toe was anesthetic. There was slight wasting of the thenar muscles, more on the right than on the left. The thyroid gland was palpable, as were the axillary and the right, suprapopliteal lymph nodes. The pretreatment biopsies revealed active lepromatous (LL) leprosy, and the LIB was 4.7. The smears gave an average BI of 4.3 and an MI of 29. The lepromin reaction measured 4 mm. at three days and 3 mm. at three weeks. The tuberculin test gave a reading of 13 mm. to 1 TU before treatment and 23 mm. after four and one half months.

Case 6. (No. 16123) Gurkha male, age 20. This patient stated that two and one half months before admission he developed a rash, first on his right arm, then on his legs and one month later behind his right ear. On examination he was severely pock-marked on his face and limbs. There were erythematous lepromatous plaques on his right forearm and behind the right ear. A moderate number of acute lepromatous plaques, some ulcerated, were present on the limbs and trunk. The conjunctivae were slightly reddened, and the suprapopliteal, axillary and inguinal lymph nodes were moderately enlarged. The plaque on the right ear was anesthetic. The tests were slightly smaller and softer than normal, and his right ulnar, lateral popliteal, superficial radial, and left and right great auricular nerves were slightly enlarged. Biopsies of the right forearm and left thigh revealed active lepromatous (LL) leprosy, with a biopsy index of 4.9. Smears gave an average MI of 40 and a BI of 4.7. The lepromin test gave a reaction of 4 mm. at three days and 0 mm. at four weeks. The tuberculin reaction (1 TU) measured 4 mm.; at the end of the trial the reaction was negative to 1 TU, and measured 10 mm. to 20 TU.

Case 7. (No. 16124) Malay male, age 14. Six months before admission, this school boy noticed a red patch on his left elbow, and then another on his left leg. Four months later red lumps appeared on his thighs and right forearm. On examination, coalescing lepromatous macules covered most of the trunk. Scattered nodules were
present on all four limbs, chiefly on the extensor surfaces. In addition there were a few small plaques with punched-out centers suggestive of old borderline lesions that had subsequently become infiltrated and lepromatous. There was fairly symmetric moderate nerve enlargement. The centers of these lesions were anesthetic. Pinprick sensation was also impaired over patchy areas of the limbs wherever the skin was more markedly infiltrated. Biopsies of the right forearm and left elbow revealed regressing lepromatous (LL) leprosy, and the LiB was 4.4. Skin smears gave an average B1 of 4.8 and an MI of 33. The lepromin test gave a reading of 4 mm, at three days and 0 mm, at three weeks. The tuberculin test was negative both on entry and at completion of the trial.

Case 8. (No. 10126) Chinese male, age 31. Two years before admission this patient developed a left foot drop and the left leg and foot became numb. Eighteen months later he noticed redness and swelling of his fingers and toes. On examination there was infiltration of his face and ears, and his fingers and toes were swollen. Scattered nodules were present, especially at the elbows, knees and heels, and over the left toes. The skin of the left calf was dry and scaly and behind the left knee the edge of an old borderline annular lesion was just visible. There was moderate nerve enlargement, especially of the left superficial radial and the left lateral popliteal nerves. The left foot drop was complete, and there was anhidrosis in the left leg and foot from below the knee. The extensor muscles of the left leg were wasted and there was also slight wasting of the intrinsic muscles of the left hand. There was patchy impairment of sensation in the right leg and the right forearm. The conjunctivae were mildly injected, the testes were smaller and softer than normal, and lymph nodes were moderately enlarged. Clinically the patient was considered to have deteriorated from borderline to lepromatous leprosy. Histologically he was graded as active lepromatous (LL) leprosy and his LiB was 5.6. His skin smears gave an average B1 of 4.5 and an MI of 29. The lepromin test was negative both at three days and at three weeks. The tuberculin test was negative to 1 TU; but gave a reading of 12 mm. to 20 TU, at the end of trial there was a 4 mm. response to 1 TU.

RESULTS

Clinical. During the four-and-one-half months-trial period, all eight patients were satisfied with their progress. None developed erythema nodosum leprosum (ENL), although Case 2 suffered from mild ulnar neuritis at four months. Case 3 was admitted with a definite lepra reaction (leprosy exacerbation), which only very slowly subsided, and his lesions were still erythematous at his final assessment.

The independent assessor rated the improvement as "moderate" in three patients (Cases 3, 4 and 8), "between slight and moderate" in three patients (Cases 5, 6 and 7), as "slight" in one patient (Case 2), and as "no change" in one (Case 1). However, the latter patient felt better and at three months reported that he could breathe more easily through his previously blocked nose. Moreover he (together with Case 8) was retained in the leprosarium for reconstructive surgery; B.663 was continued at the same dosage, and at one year the independent assessor considered that his improvement was "moderate."

No evidence was obtained from the random urinary sulfone tests that any patient was taking dispensing in addition to the B.663. On the other hand the skin color of all eight patients was consistent with their taking B.663, although the depth, and distribution of the color change varied considerably. Thus Case 3 showed only slight B.663 discoloration after four and a half months, whereas in the other seven it was far more definite. Patients with diffuse infiltration tended to develop the typical red brown color; patients with nodular leprosy usually in addition developed hypermelanosis of the localized lesions. The discoloration was first detected about six weeks after the beginning of treatment, and insidiously but steadily deepened over the ensuing three months. The final depth of pigmentation (of either variety) was often little realized, either by the doctor in clinical charge of patients or by the medi-
TABLE 1. Bacteriologic and histologic response of eight patients treated for 2½ months with B.663 (100 mgm. twice weekly).

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Bacterial index (B)</th>
<th>Logarithmic biopsy index (LIB)</th>
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<tr>
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<td>At start</td>
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<td>Average</td>
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Bacteriologic. As only lepromatous patients were admitted to the trial it is not surprising that no significant fall occurred in the B1 over four and a half months. Details of the results are given in Table 1.

Histologic. Improvement during treatment was assessed by the logarithmic biopsy index (LIB) (18-11) and by the histologic appearance of activity. For fully lepromatous (LL or LI) patients, the LIB is of little value for periods under six months, and individual results require at least two six-monthly periods of observation for accurate interpretation. Five patients showed a fall in their LIB over the four and a half months trial period; in three (Cases 3, 6 and 7) the final LIB was higher than the pretreatment figures. The results are recorded in Table 1.

In six patients the pretreatment histology showed active leprosy; in two (Cases 7 and 8) the appearances were of regressing disease. After four and a half months, the histology of Case 2 was graded as "regressing," that of Case 1 as "almost quiescent," and that of the remaining six patients as "quiescent." Thus in all eight cases the disease became less active histologically.

DISCUSSION

Hitherto the lowest dose of B.663 reported in the literature has been 100 mgm. daily for six days each week (19). Even on this dose marked pigmentation has oc-
TABLE 2. The effect of treatment with B.663 (100 mgm. twice weekly) on the morphologic index (MI) in eight previously untreated lepromatous patients.

<table>
<thead>
<tr>
<th>Case No.</th>
<th>At start</th>
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<th>3 months</th>
<th>41/2 months</th>
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<td>8</td>
<td>29</td>
<td>19</td>
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Average MI (all 8 cases X---X; Maximum MI □—□; Minimum MI O---O)
curved in paler-skinned patients. For this reason and because of the occasional occurrence of diarrhea it was essential to investigate the therapeutic activity of still smaller doses of B.663. However, until the minimum inhibitory concentration (MIC) has been titrated in the mouse foot pad and extrapolated to man, any further reduction in the dosage of B.663 in man must be decided empirically. For this reason the dose of 100 mgm. twice weekly was chosen, and the results here presented confirm that the drug is fully active at this level. The rate of clinical improvement was comparable to that obtained with DDS or with B.663 in higher dosage, and the rate of fall of the MI was equally rapid (11, 12, 13).

On the dose schedule of 100 mgm. twice weekly, no toxic effects of B.663 were detected. In particular no patient suffered from diarrhea. However, all patients developed pigmentation to a lesser or greater degree. This was usually first detected six weeks after the start of treatment, compared with one to four weeks with larger dosage (10). The depth of pigmentation attained by four and a half months was also definitely less than with 300 mgm. B.663 daily. Patients noticed and complained of the pigmentation, but were less troubled by it than patients receiving the higher dosage. Indeed one patient (Case 8) with foot drop was prepared at the conclusion of the trial to be discharged home and to work for three months still on B.663 before returning for a tendon transplant operation. On his return to the leprosarium his depth of skin pigmentation was unchanged and no sulphone was detected in his urine, confirmatory evidence that he continued on B.663 and that he was satisfied enough with his treatment not to take additional drugs. Although B.663, 300 mgm. daily, causes red discoloration of the urine (14), this was less obvious on the dose of 100 mgm. twice weekly, especially when the urine was dilute. Therefore the color of the urine on the lower dose gave less definite evidence that the drug was being taken regularly as prescribed.

Quite fortunately, no dark-skinned patients were included in the present trial. However one dark-skinned Malay patient and one Southern Indian (Dravidian) who were excluded from the trial, the first because his MI was below 55 and the second because he was histologically BL, were each given B.663, 100 mgm. twice weekly for four and a half months. At the end of this time neither patient complained of pigmented change and in fact little or none could be detected. In view of the various claims for the anti-inflammatory activity of B.663 (1, 4, 7, 18) it is also of interest to record that the latter patient developed a marked lepra reaction (leprosy exacerbation) while on B.663. The fact that none of the eight lepromatous patients included in the pilot trial developed ENL is of little significance. At Sungai Buloh ENL is rare before the MI has reached a baseline of 0-2, i.e., until after three to six months' treatment (17); and in a recent trial of low dosage DDS (11) no ENL occurred in six patients over a similar four and a half months' trial period. Furthermore there is no evidence from this small series of patients that B.663 depresses delayed hypersensitivity of the tuberculin type; indeed, in five of eight patients the tuberculin test was stronger at the completion of the trial than before treatment.

One important result of the pigmentation produced by B.663 was that the independent assessor soon realized which drug was being used in the pilot trial. Clinical assessments could therefore no longer be carried out blind. However, although there was a greater scatter in the individual assessments of clinical improvement in this trial, the average clinical improvement for the eight patients is very similar to that recorded in the immediately preceding trial of six patients receiving DDS in low dosage (11). But the pigmentation, and especially the hypermelanotic pigmentation occurring in localized lesions, made the latter stand out far more clearly than at the beginning of treatment. The impression given to the independent assessor was that the lesions had increased in size (which he had at first rationalized on the ground that the patients were putting on weight under the good conditions of the leprosarium) but that they nevertheless looked less active. Therefore in carrying out his assessments the
SUMMARY

Using a trial design previously evolved at Sungei Buloh Lepronomarium, a pilot trial was performed of B.663, in the dosage of 100 mgm. twice weekly, in eight patients with previously untreated lepromatous leprosy. The therapeutic results, as measured by clinical, bacteriologic and histologic assessment, and especially by the rate of fall of the morphologic index, were similar to those obtained with sulfone therapy or with B.603 in the dosage of 300 mgm. daily.

Although B.663 pigmentation was produced in all eight patients, it developed more slowly and was less intense than with standard dosage. Difficulties resulting from skin discoloration in assessing the clinical progress of patients on B.663 are discussed.

RESUMEN

Empleando un esquema de ensayo previamente desarrollado en Sungei Buloh Leprosarium, una prueba experimental con B.663 se llevó a efecto empleando una dosis de 100 mgm. dos veces por semana, en ocho enfermos con lepra lepromatosa, no tratados previamente. Los resultados terapéuticos según una evaluación clínica, bacteriológica y histológica y particularmente por la medida de la caída del índice morfológico, fueron similares a aquellos obtenidos con terapia sulfonílica o con B.663 en dosis de 300 mgm. diarias.

Aunque la pigmentación del B.663 se produjo en los ocho pacientes, ella se desarrolló más lentamente y fue menos intenso que con la dosis estándar. Las dificultades que resultan de la decoloración de la piel en la evaluación de la mejoría clínica de los enfermos que reciben B.663 se discuten.

REFERENCES


