Activity of KRM-1648 Alone or in Combination with both Ethambutol and Kanamycin or Clarithromycin against Mycobacterium intracellulare Infections in Beige Mice

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Mycobacterium avium complex (MAC) is a common cause of disseminated infections in patients with AIDS (4, 5, 17). The results of chemotherapy for this disease remain less than satisfactory because of the presence of multiple resistance to most of the available anticytobicidial drugs (4, 8). There is thus an urgent need for new antimicrobial agents with proven effectiveness in treating infections caused by MAC. A new rifamycin derivative, KRM-1648, 3’-hydroxy-5’-(4-isobutyl-1-piperazinyl) benzoxazinorifamycin (synthesized by Kaneka Corporation), has been reported to be highly active against MAC both in vitro (12, 14, 16) and in vivo (1, 9, 13, 15). We previously reported that the MIC of KRM-1648 at which 90% of isolates are inhibited was 1/100 that of rifampin (RFP) or less against 20 clinical MAC isolates (16). The studies reported herein were designed to evaluate the in vivo chemotherapeutic activities of KRM-1648 alone and in combination with kanamycin (KM) and ethambutol (EB) in place of RFP against M. intracellular 31F093T (7) by using a model of chronic infection in the beige mouse. In separate experiments, the in vivo activities of KRM-1648 alone and in combination with clarithromycin (CAM) were studied.

**MATERIALS AND METHODS**

Drugs. KRM-1648 was supplied by Kaneka Corporation (Takasago, Japan). RFP was obtained from Daiichi Pharmaceutical Co. (Tokyo, Japan), KM was obtained from Sigma Chemical Co. (Tokyo, Japan), EB was obtained from Lederle Japan Co. (Tokyo, Japan), and CAM was obtained from Taisho Pharmaceutical Co. (Tokyo, Japan). KRM-1648, 3’-hydroxy-5’-(4-isobutyl-1-piperazinyl) benzoxazinorifamycin was synthesized by Kaneka Corporation.

Strain 31F093T was originally isolated from the sputum of a patient with M. avium-M. intracellular infection at our institute, and it was shown to be reasonably virulent in mice (7). It was determined to be M. intracellular by DNA probe examination and was stocked frozen in our laboratory prior to the experiments. This strain was grown in 1% Ogawa egg medium. For studies of infection, this strain was grown in a modified Dubos Tween-albumin liquid medium at 37°C for 2 weeks. Treatment with the various regimens was begun 24 h after infection and was continued until the end of the experiment (12 weeks). KRM-1648 (20 mg/kg of body weight given daily), RFP (20 mg/kg given daily), and EB (20 mg/kg given daily), each finely suspended in 2.5% gum arabic-0.2% Tween 80 solution, were orally administered, and KM (20 mg/kg given every other day) dissolved in distilled water was administered subcutaneously.

In the second experiment, 8- to 10-week-old male beige mice were infected intravenously through a tail vein with approximately 10^8 viable organisms of the strain described above. Treatment was begun 24 h after infection and was continued until the end of the experiment (12 weeks).

KRM-1648, RFP, or CAM, each of which was finely suspended in 2.5% gum arabic-0.2% Tween 80 solution, was administered daily. The dose of each drug was 20 mg/kg, and each drug was administered orally.

In order to evaluate the effects of the chemotherapy regimens, three or four mice in each treatment group were killed at regular intervals after infection so that the numbers of viable bacilli in the lungs and spleens could be counted. These organs were removed aseptically and weighed, and then 10-fold dilutions of the organ homogenates were inoculated onto 7H10 agar plates to count the number of viable bacilli. In order to minimize the effect of 2.0% NaOH on the viability of the mycobacteria, the tissues were homogenized at less than 10°C, and the inoculation procedure was performed within 2 h of homogenization.

**RESULTS**

Effect of KRM-1648 in combination with KM and EB. The consecutive CFU counts in the lungs and spleens of infected beige mice treated with each drug alone or with combinations of the drugs are given in Table 1. Treatment with RFP (20 mg/kg/day) alone resulted in a negligible reduction in CFU counts in the lungs (averages) compared with those in the lungs of untreated controls through the duration of the experiment (Table 1). The CFU counts in mice treated with KM alone (20 mg/kg every other day) were essentially the same as those in mice treated with combinations of KM with the other drugs except KRM-1648 (20 mg/kg/day). The efficacy of KRM-1648 alone was comparable to those of the combination regimens of KM-EB and RFP-KM-EB. Treatment with the three-drug combination regimen KRM-1648–KM–EB reduced the...
FIG. 1. Mean log CFU counts of *M. intracellulare* 31F093T recovered from the lungs (A) and spleens (B) of infected beige mice after treatment with KRM-1648 alone or in combination with CAM. Treatment was started on day 1 after infection and was continued for 12 weeks. Each point represents the means for three to four animals. Bars represent standard deviations. KRM, KRM-1648.
CFU counts significantly more than treatment with RFP-KM-EB did \((P < 0.05)\).

Treatment with RFP alone only slightly reduced the CFU counts in the spleens (averages) compared with those in the spleens of untreated control mice (Table 1). KRM-1648 alone was as active as the combinations RFP-KM-EB and KM-EB. Although the three-drug combinations KRM-1648–KM–EB and KRM-1648–KM–EB rapidly reduced the CFU counts by week 3, thereafter, the CFU counts gradually increased. A similar tendency was observed for treatment with KRM-1648 alone.

**Effect of KRM-1648 in combination with CAM.** The CFU counts in the lungs and spleens of infected beige mice are shown in Fig. 1A and B, respectively. Treatment with RFP (20 mg/kg/day) alone only slightly reduced consecutive CFU counts in lungs compared with those in the lungs of untreated control mice (Fig. 1A). However, treatment with KRM-1648 alone (20 mg/kg/day) reduced CFU counts more than treatment with RFP did \((P < 0.05)\). The trend toward a reduction was essentially the same as that for CAM (20 mg/kg/day). The combination KRM-1648–CAM markedly reduced the CFU counts and was more active than KRM-1648 alone, CAM alone, and the combination RFP-CAM. The reduction in CFU counts in spleens observed with RFP alone was negligible compared with that in the spleens of the untreated controls. Unlike in the lung, in the spleen KRM-1648 alone was less active than CAM alone and the combination RFP-CAM. The combination KRM-1648–CAM markedly reduced the CFU counts, but the difference in CFU counts between mice treated with KRM-1648–CAM and those treated with RFP-CAM was not significant at the end of the experiment.

**DISCUSSION**

The present study confirmed the remarkable in vivo chemotherapeutic activity of KRM-1648 alone or in combination with other antimycobacterial agents against experimental *M. intracellulare* infection in beige mice. KRM-1648 was more active than RFP when each drug was used alone, but the efficacy of KRM-1648 was essentially the same as those of KM and CAM. The combination KRM-1648–KM–EB proved to be much more effective than RFP-KM-EB, achieving a rapid reduction in CFU counts in both the lungs and the spleens. The addition of KRM-1648 to the combination of KM and EB increased the activity beyond that obtained with KM-EB alone, although the difference in activity between KM alone and KM-EB was not significant. On the other hand, the combination of KRM-1648–CAM was clearly more efficacious than RFP-CAM; this difference was more pronounced for CFU counts in the lungs than for CFU counts in the spleens.

The rationale for combination chemotherapy in the treatment of mycobacterial infections includes minimization of the emergence of drug resistance and, when possible, eradication of the CFU counts in the tissue via the synergistic effects of drug combinations. Correspondingly, Kuzef and associates (7, 8) have studied the effects of various multiple-drug regimens on chronic MAC infection in mice, but even a five-drug regimen did not eradicate the CFU counts in the tissues that they tested. Potent in vivo activities of KM (11) and CAM (2, 6) against MAC infection have been reported. Recently, some investigators reported the therapeutic effects of KRM-1648 in combination with other antimycobacterial agents (1, 13). In the present study, we also confirmed the remarkable effects of combined treatment with KRM-1648–KM–EB and KRM-1648–CAM. Determination of the effects of the combination of KRM-1648 with both KM and CAM is useful in demonstrating whether an active regimen can be obtained in a model of chronic infection in mice.

In conclusion, our findings suggest that KRM-1648 is a promising candidate for combination therapy with other potent antimycobacterial drugs against MAC isolates.

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REFERENCES


