Curing *Mycobacterium ulcerans* Infection in Mice with a Combination of Rifampin-Streptomycin or Rifampin-Amikacin[▽]

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Received 6 July 2006/Returned for modification 10 August 2006/Accepted 25 October 2006

The curing activities of various durations of treatment with a combination of rifampin (RIF) and either streptomycin (STR) or amikacin (AMK) in murine *Mycobacterium ulcerans* infection were compared in two experiments. In the first experiment, treatment was begun 1 week after infection, when the inflammatory footpad lesion had not yet occurred (preventive model), and in the second experiment, treatment was begun 6 weeks after infection, when inflammatory footpad lesions were established (curative model). In the first experiment, 4 weeks of treatment with daily RIF-STR or RIF-AMK was able to postpone the occurrence of footpad lesion by 12 weeks (RIF-STR) or 17 weeks (RIF-AMK), thus demonstrating their promising bactericidal activities, but neither treatment was able to prevent the late occurrence of footpad lesions. In the second experiment, the overall cure rates, as assessed by the lack of rebound of inflammatory lesions or remultiplication of *M. ulcerans*, were only 62% after 2 weeks of treatment with RIF and an aminoglycoside and 85% after 4 weeks; but the cure rate reached 100% after 8 or 12 weeks of treatment. The cure rates were slightly higher with the AMK-containing combination than with the STR-containing combination, but the difference was at the limit of significance ($P = 0.07$). These results show that in the murine model of Buruli ulcer, 8 weeks is the optimal duration of treatment with a combination of RIF and an aminoglycoside.

Buruli ulcer, or *Mycobacterium ulcerans* infection, is a major emerging disease and the third most common mycobacterial disease in humans after tuberculosis and leprosy. It has been reported from over 30 countries in Africa, Southeast Asia, and South America and from Australia (9–11). Until recently, the standard treatment has been wide surgical excision followed by skin grafting (10), but surgical treatment is often not available in rural areas where the disease is endemic. Therefore, development of an effective and affordable drug treatment is a research priority for the control of Buruli ulcer (9). The recent demonstration of the promising therapeutic effects of the combination of rifampin (RIF) and streptomycin (STR) in human clinical trials (5; A. Chauty, 8th Annu. World Health Organization Advisory Group Meet. Buruli ulcer, 2005) is a major breakthrough in the treatment of Buruli ulcer, representing the first evidence that preulcerative or small to moderately sized Buruli ulcers may be cured by chemotherapy without surgery (Chauty, 8th Annu. World Health Organization Advisory Group Meet. Buruli ulcer). Nevertheless, the optimal duration of treatment with RIF-STR remains to be determined. Furthermore, the combination of RIF plus amikacin (AMK), another aminoglycoside, also displays powerful bactericidal activity against *M. ulcerans* in mice (3, 6), but the optimal duration of treatment with this combination is also unknown.

The objectives of the study were to compare the activities of the combinations of RIF-STR and RIF-AMK in a preventive

and curative model of *M. ulcerans* infection in mice and to determine the minimal duration of treatment required to obtain a high cure rate.

MATERIALS AND METHODS

Infection of mice with *M. ulcerans* isolate. Strain Cu001 was isolated from a patient with Buruli ulcer in Côte d'Ivoire and was maintained in our laboratory through regular passage in mouse footpads. This isolate has been used in several experiments for testing of the in vivo activities of various antimicrobial agents against *M. ulcerans* (1, 3, 4, 6). The MICs of RIF, STR, and AMK against isolate Cu001 are 2, 0.5, and 1 $\mu\text{g/ml}$, respectively, and do not differ significantly from the MIC₉₀s of the three antimicrobial agents for 28 other clinical isolates of *M. ulcerans* (6).

Female BALB/c mice of 4 weeks of age were purchased from the Janvier Breeding Center, Le Genest-Saint-Isle, France. In conducting the in vivo experiments, the Laboratoire de Bactériologie-Hygiène strictly followed the official instructions (A 75-13-01, issued by the Direction Départementale des Services Vétérinaires de Paris and the Préfecture de Police de Paris) for the appropriate use of animals. In each experiment, the left hind footpad of each mouse was inoculated subcutaneously with 0.03 ml of an *M. ulcerans* suspension. The inoculum per footpad contained 4.87 log₁₀ acid-fast bacilli (AFB), as assessed by the microscopic counting method (8), in the first experiment, and 5.04 log₁₀ AFB or 4.20 log₁₀ CFU in the second experiment.

Treatment of mice. The following compounds were provided by their manufacturers: RIF, Aventis, Paris, France; AMK, Bristol-Myers-Squibb, Paris, France; and streptomycin (STR), Panpharma, Fougères, France. RIF was suspended in 0.05% agar-distilled water and given by oral gavage; STR or AMK was diluted with normal saline and administered by subcutaneous injection. All antimicrobial agents were administered once daily for 5 days per week. As in previous experiments, the daily dosages were RIF at 10 mg/kg of body weight and STR or AMK at 150 mg/kg.

Occurrence of inflammatory footpad lesions in mice treated with RIF-STR or RIF-AMK for 4 weeks in a preventive model (first experiment). Fifty-seven mice were inoculated. One week after infection, when the lesion index (see "Scoring of lesion index" below) of the inoculated footpads was still 0, 10 mice were killed for determination of the pretreatment (day 0) values of the mean numbers (log₁₀) of AFB and CFU per footpad; the remaining 47 mice were randomly allocated among three groups: one untreated control of 17 mice and two treated

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[▽] Published ahead of print on 13 November 2006.

groups of 15 mice each. Treatment with either RIF-STR or RIF-AMK was begun immediately after randomization and lasted 4 weeks. By the end of 4 weeks of treatment, all 17 mice from the untreated control group and 5 mice from each treated group were killed for the enumeration of AFB and CFU per footpad. The remaining 10 mice from each group were held without treatment for an additional 28 weeks to detect the time of occurrence of footpad lesions.

Determination of cure rate of *M. ulcerans* infection in mice after various durations of treatment with RIF-STR or RIF-AMK in a curative model (second experiment). Four hundred twenty-four mice were inoculated. Six weeks later, at which time inflammatory swelling with a "lesion index" of 2 or 3 was observed in all inoculated footpads (see "Scoring of lesion index" below), 10 mice were killed for measurement of the pretreatment (day 0) values of the mean number of CFU per footpad. The remaining 414 mice were randomly allocated among 12 groups: an untreated control group of 34 mice; three groups of 20 mice each treated with RIF, AMK, or STR alone, respectively, for 12 weeks; and eight groups of 40 mice each treated with RIF-STR or RIF-AMK for either 2, 4, 8, or 12 weeks. Treatment was begun immediately after randomization, as described previously (3, 6). Because they were seriously ill, 20 untreated control mice were killed by the end of 2 weeks and the 14 remaining mice were killed by the end of 4 weeks. For the groups treated with RIF, AMK, or STR alone, all mice were killed at the end of 12 weeks of treatment. Among the 40 mice in each of the eight groups treated with RIF-STR or RIF-AMK for various durations, 20 mice were killed at the end of treatment and the remaining 20 mice were held without treatment for an additional 28 weeks, with the aim of detecting a rebound of the lesion index.

Assessments of severity of infection and effectiveness of treatment. Two indicators were used to assess the severity of infection and the effectiveness of treatment: (i) the lesion index of the inoculated footpads and (ii) the mean number ($\log_{10}$) of CFU per footpad.

(i) **Scoring of lesion index.** For both experiments, the mice were examined for determination of the lesion index of the footpad once weekly. On the basis of the presence and the degree of inflammatory swelling of the inoculated footpad, the lesion index was scored from 0 to 5, as follows: 0, the footpad appeared normal; 1, the footpad showed slight swelling but no clear inflammatory reaction; 2, definite inflammatory swelling was limited to the footpad; 3, inflammatory swelling was extended to the whole hind foot; 4, inflammatory swelling was extended to the whole limb; and 5, inflammatory swelling of the whole limb was accompanied by a deteriorating general condition. The average lesion index (ALI) was calculated by averaging the lesion index for each group (3).

(ii) **Enumeration of CFU in footpads.** The tissues of the inoculated footpads were removed aseptically at the time that the mice were killed and were homogenized in Hanks' solution in a final volume of 2 ml. The suspensions were plated on Löwenstein-Jensen medium and incubated at 30°C up to 90 days. For the untreated control mice, the tissue suspension was serially diluted in 10-fold steps, and 0.1 ml of each of three appropriate dilutions was cultivated in triplicate. For the treated mice, in the first experiment, 0.1 ml of tissue suspension diluted 10^{-1} was cultivated in triplicate. In the second experiment, the entire volume (2 ml) of the undiluted tissue suspension from each footpad was cultivated; for the mice killed at the end of 2 weeks treatment, however, 0.1 ml of a suspension diluted 10^{-2} was cultivated in triplicate. The lower limit of the CFU count per footpad varied according to the dilution of the footpad suspension and to the number of medium tubes plated with the suspension; when the entire volume (2 ml) of undiluted tissue suspension from each footpad was plated in 10 tubes of Löwenstein-Jensen medium, the lower limit of the CFU count was a single colony.

Interpretation of results. (i) **Preventive model (first experiment).** In the preventive model (the first experiment), the intervals between infection and the occurrence of footpad lesions in treated and untreated mice were expressed by the "median time to occurrence of a footpad lesion," i.e., the interval required to develop a lesion index of ≥ 2 in 50% of the inoculated footpads in the group (1, 4), as determined by the Kaplan-Meier method. In comparisons of the median times between the treated and the untreated control groups, a regimen was considered bactericidal if the median time for the treated group was significantly longer than that for the control group plus the duration of treatment; a regimen was considered bacteriostatic if the median time for the treated group was significantly longer than that for the untreated control group but was not significantly longer than that for the untreated control group plus the duration of treatment; and a regimen was considered inactive if the median time for the treated group did not differ significantly from that for the untreated control group.

(ii) **Determination of cure rate in curative model (second experiment).** For the determination of the cure rate in the curative model (the second experiment), inflammatory lesions with a lesion index of 2 or 3 had already occurred in all inoculated footpads before treatment began. The cure rate was assessed on the basis of the results obtained during follow-up. During the 28 weeks of posttreat-

ment follow-up, the inoculated footpads were examined weekly to assess the reoccurrence of the inflammatory footpad, defined by a lesion index rebound to ≥ 3 (clinical relapse). Mice with a reoccurrence of lesions were killed, and the tissue suspension from the inoculated footpads was cultivated as described above in "Enumeration of CFU in footpads" to confirm a relapse. By the end of 28 weeks of follow-up, all mice with no reoccurrence of swelling were also killed, and the tissue suspension from the inoculated footpad was systematically cultivated; it was concluded that a bacteriological relapse had occurred if the culture was positive, even if there was no rebound of the lesion index in the footpad.

Statistical analysis. Survival analysis, which was performed by using the occurrence (or reoccurrence) of footpad lesion as the end point, was done by the Kaplan-Meier method (7). The log rank test was used to determine the level of statistical significance when the survival curves for the different groups were compared. The Mann-Whitney test was used to compare AFB and CFU counts, and Fisher's exact test was used to compare the proportions. *P* values are two tailed, and a *P* value of ≤ 0.05 was considered statistically significant.

RESULTS

Preventive model (first experiment). No mice in the untreated control group died, most likely because all untreated control mice were killed at 5 weeks after inoculation, whereas death due to *M. ulcerans* infection in untreated controls usually begins at 8 to 12 weeks after inoculation (1, 3, 4, 6). One mouse in the group treated with RIF-STR died in the first week of treatment because of a gavage-related accident.

(i) **Enumeration of AFB and CFU per footpad at end of treatment.** When treatment began, the mean number ($\log_{10}$) of AFB per footpad was 4.77 ± 0.25 . By the end of 4 weeks of treatment, the mean numbers of AFB and CFU were 6.45 ± 0.37 and 5.94 ± 0.21 , respectively, for the untreated control group but were 5.01 ± 0.28 and <1.85 , respectively, for the group treated with RIF-AMK and 5.08 ± 0.35 and $<2.00 \pm 0.34$, respectively, for the group treated with RIF-STR. The mean numbers of CFU for the treated groups were significantly smaller than that for the untreated control group ($P < 0.001$). The mean numbers of AFB for the treated groups did not differ significantly from the pretreatment value ($P > 0.05$), demonstrating that while the treatments did prevent the increase in the numbers of AFB over the 4-week period, the AFB count is not a sensitive indicator of the effectiveness of chemotherapy for *M. ulcerans*, as shown previously (3).

(ii) **Median time to occurrence of footpad lesion.** At the time of the start of treatment, 1 week after inoculation, all footpads were free of lesions (lesion index, 0). By the end of the fourth week of treatment (i.e., 5 weeks after inoculation), while all the footpads ($n = 15$) in each treated group remained free of lesions, definite inflammatory swelling of the footpads (lesion index, 2) was observed in all 17 untreated control mice.

During the posttreatment follow-up, swelling footpads with a lesion index of 2 were observed in all 9 mice that had been treated with the RIF-STR combination and in 6 of 10 mice that had been treated with the RIF-AMK combination. All the swelling footpads were *M. ulcerans* culture positive, and the mean numbers ($\log_{10}$) of CFU per swelling footpad were 5.20 ± 1.23 and 5.67 ± 0.45 for the groups treated with RIF-AMK and RIF-STR, respectively; i.e., both values were significantly greater than the values for the corresponding groups at the end of treatment ($P = 0.004$ and $P = 0.002$, respectively), indicating that the inflammatory swelling of the footpads was due to the multiplication of *M. ulcerans*. For four mice that were treated with RIF-AMK but that had no swelling, the footpad tissue cultures were negative at the end of the follow-up.

FIG. 1. Cumulative probability (Kaplan-Meier method) of not having an inflammatory footpad lesion in various groups of mice (first experiment).

As shown in Fig. 1, the median time of occurrence of footpad lesions was 17 weeks after infection (i.e., 12 weeks after the cessation of treatment) in the group treated with RIF-STR, with a 95% confidence interval of 16 to 18 weeks, and was 22 weeks after infection (i.e., 17 weeks after the cessation of treatment) in the group treated with RIF-AMK, with a 95% confidence interval of 18 to 26 weeks. The median times for the two treated groups were significantly longer than that for the untreated control (4 weeks) plus the duration of treatment (4 weeks) ($P < 0.05$), indicating that both treatments displayed significant bactericidal effects against *M. ulcerans*. Furthermore, the Kaplan-Meier curve drawn from the data for the RIF-AMK-treated mice was significantly more favorable than that drawn for the data for the RIF-STR-treated mice ($P = 0.01$), suggesting that the former combination has more potent activity.

Curative model (second experiment). During the second experiment, no deaths were observed among untreated or treated mice, although two mice treated with RIF-STR died due to a gavage-related accident.

(i) Evolution of ALI during treatment. At the beginning of treatment, the ALI was 2.7 ± 0.6 ($n = 424$). As shown in Table 1, the ALI for the untreated control mice rapidly increased to 3.6 ± 0.6 ($n = 34$) by the end of 2 weeks of treatment. In contrast, the ALIs for mice treated with RIF-STR and RIF-AMK gradually and progressively declined from the pretreatment value after 2, 4, and 8 weeks of treatment and remained at low levels for up to 12 weeks. More precisely, the proportion of treated mice showing higher lesion index scores (i.e., 3 or 4)

was reduced from 60% at day 0 to 23% at 2 weeks, 2% at 4 weeks, 1% at 8 weeks, and 0% at 12 weeks.

(ii) Evolution of mean number ($\log_{10}$) of CFU per footpad in various groups of mice. At the beginning of treatment, the mean number ($\log_{10}$) of CFU per footpad was 5.74 ± 0.49 . By the end of the initial 2 weeks of treatment, while the mean number of CFU per footpad increased significantly to 6.12 ± 0.42 ($P = 0.03$) for the untreated control mice, the mean numbers for mice treated with RIF alone, STR alone, AMK alone, RIF-STR, and RIF-AMK were 4.13 ± 0.43 , 4.13 ± 0.59 , 3.92 ± 0.24 , 2.84 ± 0.01 , and 2.93 ± 0.19 , respectively; all values were significantly less than the pretreatment value ($P < 0.001$). The values did not differ significantly between the mice that had been treated with either aminoglycoside, with either monotherapy, or with either combination with RIF. The mean number of CFU among mice treated with RIF-STR or RIF-AMK was significantly ($P < 0.001$) lower than that among mice treated with the individual component administered as monotherapy, suggesting that the combination of RIF with an aminoglycoside has a synergistic effect. As expected (6), at the end of 4, 8, or 12 weeks of treatment with either RIF-STR or RIF-AMK, all the inoculated footpads were culture negative.

(iii) Follow-up after end of treatment. As shown in Fig. 2 and 3 and Table 2, the outcomes of the follow-up were quite different among the groups that had been treated with RIF and an aminoglycoside for various durations. After the cessation of treatment, the lesion index continued to decrease among the groups that had been treated for only 2 or 4 weeks, whereas such a postantibiotic effect was not observed among the groups that had been treated for 8 or 12 weeks, most likely because the lesion index had already decreased to a relatively low level by the end of treatment with longer durations. During the later stage of follow-up, which ranged from 20 to 28 weeks after the cessation of treatment, a rebound of the lesion index to ≥ 3 was observed among 8 of 20, 5 of 20, 4 of 20, and 1 of 20 mice that had been treated with RIF-STR for 2 weeks, RIF-AMK for 2 weeks, RIF-STR for 4 weeks, and RIF-AMK for 4 weeks, respectively. Among these four groups, the mean numbers ($\log_{10}$) of CFU for the footpads with rebounding lesion indices were 6.18 ± 0.14 , 5.49 ± 0.37 , 3.12 ± 1.74 , and 5.95, respectively, which were significantly greater than the CFU counts by the end of treatment for the same groups, thus demonstrating that the rebound of the lesion index was due to the remultiplication of *M. ulcerans*. As shown in Table 2, in addition to the clinical relapses, one bacteriological relapse (i.e., no rebound of the lesion index but *M. ulcerans* culture positivity by the end of follow-up) was detected in each of the groups that had been treated with RIF-STR or RIF-AMK for 2 weeks and RIF-STR for 4 weeks. In contrast, no clinical or bacteriological relapse

TABLE 1. ALI per inoculated footpad for various groups of mice (second experiment)

Treatment	Mean ALI $\pm$ SD (no. of mice) for the following duration of treatment:				
	Day 0	2 wk	4 wk	8 wk	12 wk
None (untreated controls)	2.7 ± 0.6 (424)	3.6 ± 0.6 (34)	5.0 ± 0 (14)		
RIF-STR		2.2 ± 0.5 (159)	2.1 ± 0.6 (120)	1.3 ± 0.5 (80)	1.2 ± 0.5 (40)
RIF-AMK		2.2 ± 0.5 (160)	1.9 ± 0.5 (120)	1.4 ± 0.6 (80)	1.4 ± 0.5 (40)

FIG. 2. ALIs over time for footpads of mice treated with RIF or AMK alone or with the combination RIF-AMK for various durations (second experiment). †, the mice were killed before spontaneous death due to *M. ulcerans* infection.

was observed among mice that had been treated with either RIF-STR or RIF-AMK for 8 or 12 weeks. In summary, by combining the results for mice that had been treated with RIF-STR and RIF-AMK for the same durations, the cure rate (i.e., the proportion of mice without a clinical or a bacteriological relapse after 28 weeks of posttreatment follow-up) of *M. ulcerans* infection obtained by treatment with RIF and an aminoglycoside increased from 62% after 2 weeks of treatment to 85% after 4 weeks of treatment and reached 100% after 8 or 12 weeks of treatment (Table 2). The cure rates were higher with the AMK-containing combination, 70% and 95% after 2 and 4 weeks of treatment, respectively, whereas with the STR-containing combination they were 55% and 75%, respectively (Fig. 4). However, the difference was at the limit of significance ($P = 0.07$).

DISCUSSION

In mycobacterial diseases, after identification of an effective chemotherapy regimen, the most important tasks are determination of the adverse effects and the curing activity, as assessed by the relapse rate. Measurement of the relapse rate after the cessation of treatment is the only proven way to assess the long-term efficacy of a chemotherapy regimen.

The combination of RIF and STR has only recently been tested for use for the treatment of Buruli ulcer in humans (5; Chauty, 8th Annu. World Health Organization Advisory Group Meet. Buruli ulcer). The only available information about the rates of relapse of Buruli ulcer after treatment with

a RIF-aminoglycoside combination was obtained from a clinical trial carried out in Pobé, Benin (Chauty, 8th Annu. World Health Organization Advisory Group Meet. Buruli ulcer). Of the 124 Buruli ulcer cases which had been treated for 8 weeks with daily RIF-STR, with or without surgical excision, only 3 (2.4%) relapsed during the first year of posttreatment follow-up (Chauty, 8th Annu. World Health Organization Advisory Group Meet. Buruli ulcer). No relapse data for shorter or longer durations of treatment with RIF-STR are available. Moreover, no data about the relapse rate after treatment with RIF-AMK are available either for humans or from animal models, although this regimen exhibited promising bactericidal activity in mice (1, 3, 6). While the preliminary data about the relapse rate obtained for humans after 8 weeks of treatment with RIF-STR are very encouraging, the observation must be confirmed by additional studies. Furthermore, to determine the optimal duration of treatment with RIF and an aminoglycoside, the relapse rates after various durations of treatment and treatment with various aminoglycosides should be compared. Nevertheless, for practical reasons, one can hardly compare the relapse rates for more than two or three treatment regimens in a single human clinical trial. For ethical reasons, it is also difficult to assess in human clinical trials the activity of a shorter duration of treatment. In experiments with mice, however, one may compare several regimens and provide unique information about relapse rates otherwise not available from human clinical trials.

FIG. 3. ALIs over time for footpads of mice treated with RIF or STR alone or with the combination RIF-STR for various durations (second experiment). †, the mice were killed before spontaneous death due to *M. ulcerans* infection.

We have therefore compared the relapse rates of the combinations RIF-STR and RIF-AMK in two experiments with mice. In the first experiment, a treatment of 4 weeks was begun 1 week after infection, before the occurrence of inflammatory footpad lesion (preventive model). In the second experiment, treatment for either 2, 4, 8, or 12 weeks was begun when definite inflammatory swelling (lesion index 2 or 3) was observed in all inoculated footpads (curative model). In the first experiment, the occurrence of inflammatory lesions was postponed for a long period of time (12 to 17 weeks), but eventually, inflammatory footpad lesions occurred in the majority of mice that had been treated with either RIF-STR or RIF-AMK, demonstrating that 4 weeks of treatment is not long enough to kill all the bacilli and to prevent the late development of infection. In this model the proportion of mice that developed footpad lesions was slightly higher for mice treated with RIF-STR than for those treated with RIF-AMK (9/9 versus 6/10). Moreover, after 4 weeks of treatment, the median time to the

occurrence of footpad lesions was significantly longer in the group treated with RIF-AMK (17 weeks) than in the group treated with RIF-STR (12 weeks) ($P = 0.01$), suggesting that the former combination has a higher level of activity.

In the second experiment, the cure rates of *M. ulcerans* infection after treatment with RIF-STR were slightly lower (55% after 2 weeks and 75% after 4 weeks) than those after treatment with RIF-AMK (70% and 95%, respectively), but the difference was at the limit of significance ($P = 0.07$). The fact that the average lesion index continued to decrease after

FIG. 4. Cumulative probability (Kaplan-Meier method) of not having rebound of footpad lesion (clinical relapse) during the 28-week posttreatment follow-up (second experiment).

TABLE 2. Cure rate of *M. ulcerans* infections after various durations of treatment with combination of RIF and an aminoglycoside (second experiment)

Treatment	Cure rate (no. of mice cured/no. of mice treated [%]) for the following duration of treatment:			
	2 wk	4 wk	8 wk	12 wk
RIF-STR	11 ^a /20 (55)	15 ^a /20 (75)	19/19 (100)	19/19 (100)
RIF-AMK	14 ^a /20 (70)	19/20 (95)	20/20 (100)	20/20 (100)
Total	25/40 (62.5)	34/40 (85)	39/39 (100)	39/39 (100)

^a One bacteriological relapse, i.e., no rebound of lesion index but mouse *M. ulcerans* culture positivity by the end of follow-up, was observed in each of these groups.

the end of a treatment for only 2 or 4 weeks and reached the same low values as those for the groups treated for 8 weeks or longer suggests a prolonged postantibiotic effect.

The most important result of the study was that the overall rates of cure of *M. ulcerans* infection with the combination of RIF and an aminoglycoside in the curative model were 100% after 8 or 12 weeks of treatment but 85% after 4 weeks of treatment and only 62% after 2 weeks of treatment. Therefore, in the curative model, as shown for the preventive model, treatment for 4 weeks is not long enough to cure *M. ulcerans* infection in mice. After 4 weeks of treatment with RIF and an aminoglycoside, the rates of occurrence of inflammatory foot-pad lesions in the first experiment were higher than the rates of relapse in the second experiment. The difference was probably due to the methodological differences between the preventive model and the curative model.

If one arbitrarily considers that the upper limit of an acceptable relapse rate for Buruli ulcer is 5%, based on the story for tuberculosis treatment (2), the rate of relapse of *M. ulcerans* infection in mice after 4 weeks of treatment with the combination RIF and an aminoglycoside (either STR or AMK) is unacceptably high. In contrast, no relapse was observed in mice after 8 or 12 weeks of treatment with the same regimen, which is consistent with the finding of a very low relapse rate after 8 weeks of treatment with RIF-STR among small to moderately sized Buruli ulcers in a recent human clinical trial (Chauty, 8th Annu. World Health Organization Advisory Group Meet. Buruli ulcer). Consequently, 8 weeks appears to be the optimal duration of treatment with RIF and an aminoglycoside for Buruli ulcer, as proposed by the World Health Organization (12).

In our earlier experiments with mice, the bactericidal activity against *M. ulcerans* did not differ significantly between monotherapy with STR and that with AMK, nor did it differ significantly between the combination of RIF and STR and the combination of RIF and AMK (1, 6). Even though the results of the present studies suggest that RIF-AMK is slightly more active than RIF-STR in curing *M. ulcerans* infection in mice, the difference was only marginal ($P = 0.07$). Therefore, from a therapeutic point of view, STR and AMK are interchangeable for the treatment of Buruli ulcer. Because AMK is far more expensive than STR, there seems to be no justification for using AMK for the treatment of Buruli ulcer in field programs,

for which cost is one of the most important factors in choosing a treatment. On the other hand, because AMK does not show cross-resistance with STR in mycobacteria, it should be useful for the treatment of patients whose *M. ulcerans* isolates are resistant to STR, as in patients with tuberculosis.

ACKNOWLEDGMENTS

This investigation was funded by the Association Française Raoul Follereau, Paris, France, and by the Ministère de la Recherche, France (grant UPRES EA 1541).

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