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In Vivo Antileprosy Activity of the Newly Synthesized Benzoxazinorifamycin, KRM-1648[1]

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Previously, we found that the newly synthesized benzoxazinorifamycin, KRM-1648 (Kaneka Corporation, Hyogo, Japan), had excellent *in vitro* antimycobacterial activity ([5]) and potent therapeutic efficacy against experimental murine infections with *Mycobacterium avium* complex ([7]) and with *M. marinum* ([9]). KRM-1648 also was reported to be highly efficacious against *M. tuberculosis* infection in mice ([4]). Since KRM-1648 has higher antimycobacterial activity than rifampin ([4, 5, 7, 9]), it could prove to be preferable to replace rifampin, currently a component of the most reliable multidrug therapy [diaminodiphenyl sulfone (DDS) + clofazimine + rifampin] used in the clinical control of leprosy patients, with KRM-1648. Thus, in this study we investigated the therapeutic efficacy of KRM-1648 against *M. leprae* infection induced in athymic nude mice.

MATERIALS AND METHODS

Mice. Female, BALB/c, athymic nude (nu/nu) mice were purchased from Japan Clea Co., Osaka, Japan. They were bred under sterile conditions using a vinyl isolator.

Organisms. *M. leprae* Thai-53 were harvested from the infected foot pads of nude mice (donated by Dr. Kohsaka, National Institute for Leprosy Research, Tokyo).

Antimicrobial agents. The drugs tested were: KRM-1648 (Kaneka) and rifampin (Daiichi Pharmaceutical Company, Tokyo, Japan).

Experimental infection. Female, BALB/c nude mice (5 weeks old) were infected subcutaneously with 1×10^6 *M. leprae* in the left hindfoot pad. KRM-1648 and rifampin were emulsified in 0.1 ml of 2.5% gum arabic-0.2% Tween 80 solution and given to the mice by gavage once daily, six times per week, for 50 days from day 31 to day 80 after infection. All mice were observed for swelling in the left hindfoot pad. On day 360 the mice were killed, and the number of acid-fast bacilli (AFB) in the left hindfoot pad were measured according to a modified method of Shepard ([6]). Foot pad thickness was measured using dial-gauge calipers (Kagaku Kyoeisha Co., Tokyo, Japan).

RESULTS

Table 1 compares the *in vivo* antileprosy activity of KRM-1648 with rifampin. The infected mice were given each drug at the dose of 0.1 mg/mouse/day. Log decreases of 3.9 and 1.2 were recorded compared to untreated control animals in the number of organisms recovered from the infected foot pads at day 360 in mice given KRM-1648 and rifampin, respectively. There was a statistically significant difference between the value for KRM-1648 and that for rifampin ($p < 0.01$). Therefore, when given in the same doses the therapeutic activity of KRM-1648 against leprosy is higher than rifampin.

Table 2 shows the dose-dependent therapeutic effect of KRM-1648. Even at the lowest dose, 0.001 mg/mouse/day, KRM-1648 significantly decreased (ca 0.6-log) the number of AFB recovered from the infected foot pad on day 360 ($p < 0.05$). The other doses, 0.005 and 0.01 mg/mouse/day, caused a 1.0- and a 2.2-log decrease, respectively. Thus, KRM-1648 exhibited a dose-dependent antileprosy action. It also markedly inhibited foot pad swelling due to the infection in a dose-dependent manner.

Table 3 indicates the therapeutic efficacy of KRM-1648 in mice treated with a drug-containing diet prepared by Japan Clea. When mice were fed a diet containing 0.00004%–0.0004% of KRM-1648 (equivalent to doses of 0.0012 mg–0.012 mg/mouse/day) from day 31 to day 80, 2.4- to 4.2-log decreases were seen in the number

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TABLE 1. *Therapeutic efficacy of KRM-1648 and rifampin against* M. leprae *infection in mice.*[a]

Group	Inoculum size	Acid-fast bacilli harvested			Foot pad thickness (mm)
		AFB/FP[b]	Mean	Log (AFB/FP)[c]	
Control	1×10^6	1.6×10^8 4.8×10^9 4.6×10^9 2.5×10^9 8.8×10^8	2.6×10^9	9.18 ± 0.62	6.25 ± 1.30
KRM-1648	1×10^6	7.9×10^5 3.9×10^5 1.1×10^6 3.6×10^5	5.6×10^5	5.27 ± 1.16	2.88 ± 0.16
Rifampin	1×10^6	3.7×10^8 3.8×10^9 2.3×10^7 1.8×10^7 8.5×10^6	8.4×10^8	7.94 ± 1.11	4.56 ± 0.92

[a] Mice were given each drug by gavage at the dose of 0.01 mg/mouse/day as described in Materials and Methods. Mice were killed on day 360.

[b] Acid-fast bacilli/foot pad.

[c] The mean ± S.D. (N − 1) is indicated. There were statistically significant differences between the control and KRM-1648 ($p < 0.005$, Student's t test) and between KRM-1648 and rifampin ($p < 0.01$).

of AFB in the infected foot pad on day 360. Thus, the therapeutic efficacy of KRM-1648 may be increased by feeding mice the drug-containing diet (as compared to the gavage schedule of once daily, six times per week). It is difficult to make a definite conclusion on this point because there were differences in conditions between the two experiments in Tables 2 and 3.

DISCUSSION

A newly developed benzoxazinorifamycin, KRM-1648, exhibited excellent *in vivo* antileprosy activity. The therapeutic efficacy of KRM-1648 was significantly higher on an equal weight basis compared to that of rifampin. The higher activity of KRM-1648 seems mainly to be due to a higher inherent antimicrobial activity against *M. leprae,* since its pharmacokinetics are similar to rifampin in that they both attained similar blood levels ([7]). Furthermore, it should be emphasized that the toxicity of KRM-1648 is similar to or lower than that of rifampin ([7]). Therefore, it may be advantageous to use KRM-1648 instead or rifampin in the multidrug therapy of leprosy along with dapsone and clofazimine and other agents having potent antileprosy activity, including clarithromycin ([1, 2, 8]). However, more research related to the pharmacokinetics and toxicity of KRM-1648 is needed before large-scale clinical application can be recommended.

When mice were fed with a KRM-1468-containing diet, the drug exhibited a higher efficacy against leprosy than when given once daily by gavage. Although the precise reason is unknown, this mode of administration might considerably improve the pharmokinetics (absorption, tissue distribution and retention) of KRM-1648. Since the results of Tables 2 and 3 were derived from different experiments, it is difficult to arrive at a conclusion on this point. More detailed studies are needed with respect to administration protocols of this drug, which may be superior to rifampin in leprosy multidrug therapy if the toxicity and cost are comparable to rifampin.

SUMMARY

The *in vivo* anti-*Mycobacterium leprae* activity of the newly synthesized benzoxazinorifamycin, KRM-1648, was studied. KRM-1648 was given orally to athymic nude mice, infected subcutaneously with *M. leprae* in the hindfoot pad, at doses between 0.001 and 0.01 mg of the drug/mouse/day six times per week, from day 31 to day 80. KRM-1648 administration markedly suppressed bacterial growth in the foot pads for

TABLE 2. *Dose-dependency of the therapeutic efficacy of KRM-1648 against* M. leprae *infection in mice.*[a]

Agent	Dose (mg/mouse/day)	Acid-fast bacilli harvested			Foot pad thickness (mm)
		AFB/FP[b]	Mean	Log (AFB/FP)[c]	
None	0	2.6×10^8 1.4×10^8 1.2×10^7 1.3×10^8 2.0×10^8	1.5×10^8	8.01 ± 0.53	6.16 ± 0.87
KRM-1648	0.001	1.8×10^7 1.2×10^7 2.7×10^7 6.1×10^7 2.2×10^7	2.8×10^7	7.38 ± 0.26	4.00 ± 0.60
KRM-1648	0.005	1.3×10^7 2.6×10^7 6.0×10^6 6.5×10^6	1.3×10^7	7.03 ± 0.30	3.90 ± 0.66
KRM-1648	0.01	4.3×10^5 1.0×10^6 1.8×10^6 3.6×10^5	9.0×10^5	5.86 ± 0.33	3.30 ± 0.44

[a] Mice were given indicated doses of KRM-1648 by gavage as described in Materials and Methods. Mice were killed on day 360.

[b] Acid-fast bacilli/foot pad.

[c] The mean ± S.D. (N − 1) is indicated. There were statistically significant differences between the control (none) and either KRM-1648 at 0.001 mg ($p < 0.05$), KRM-1648 at 0.005 mg ($p < 0.025$) or KRM-1648 at 0.01 mg ($p < 0.005$).

TABLE 3. *Dose-dependency of the therapeutic efficacy of KRM-1648 in mice fed with the drug-containing diet against* M. leprae *infection induced in mice.*[a]

Agent	Concentration in the feed (%)	Acid-fast bacilli harvested			Foot pad thickness (mm)
		AFB/FP[b]	Mean	Log (AFB/FP)[c]	
None	0	3.4×10^8 8.9×10^8 3.4×10^8 4.5×10^7	4.1×10^8	8.44 ± 0.55	5.85 ± 0.34
KRM-1648	0.00004 [0.0012 mg/mouse]	6.8×10^5 6.0×10^6 5.3×10^5 9.4×10^5	2.0×10^6	6.08 ± 0.48	3.95 ± 0.20
KRM-1648	0.0002 [0.006 mg/mouse]	6.3×10^4 9.4×10^4 1.5×10^5 1.4×10^5 4.7×10^4	9.7×10^4	4.94 ± 0.22	3.18 ± 0.16
KRM-1648	0.0004 [0.012 mg/mouse]	9.3×10^4 9.0×10^3 3.6×10^3 2.3×10^4	3.2×10^4	4.21 ± 0.60	3.28 ± 0.18

[a] KRM-1648-containing diet was given to mice daily from day 31 to day 80 after infection; mice were killed on day 360.

[b] Acid-fast bacilli/foot pad.

[c] The mean ± S.D. (N − 1) is indicated. There were statistically significant differences between the control (none) and either KRM-1648 at 0.00004% ($p < 0.005$), KRM-1648 at 0.0002% ($p < 0.005$), or KRM-1648 at 0.0004% ($p < 0.005$).

360 days. KRM-1648 given daily at the dose of 0.01 mg/mouse elicited a 2–4-log decrease in the number of acid-fast bacilli. The therapeutic effects of KRM-1648 were significantly higher than that of rifampin when both drugs were given in the same dosage. Moreover, when mice were fed a KRM-1648-containing diet (0.00004%–0.0004%), the drug displayed an even higher efficacy against *M. leprae* infection, causing an almost 4-log decrease in the number of leprosy bacilli in the infected foot pad compared to untreated controls.

RESUMEN

Se estudió el efecto *in vivo* de la droga recién sintetizada benzoxazinorifamicina, KRM-1648, contra el *Mycobacterium leprae*. La droga se administró oralmente a ratones atímicos infectados subcutáneamente con *M. leprae* en la almohadilla plantar, a dosis entre 0.001 y 0.01 mg/ratón/día, seis veces por semana, del día 31 al día 80. La administración de KRM-1648 suprimió marcadamente el crecimiento bacteriano en la almohadilla plantar durante 360 días. La KRM-1648 administrada oralmente a la dosis de 0.01 mg/ratón, condujo a una disminución de 2–4 log en el número de bacilos. Los efectos terapéuticos de KRM-1648 fueron significativamente mayores que aquellos de la rifampina cuando ambas drogas se administraron en las mismas dosis. Además, cuando los ratones fueron alimentados con una dieta conteniendo KRM-1648 (0.00004%–0.0004%), el efecto de la droga contra el *M. leprae* fue todavía mayor, causando una disminución de casi 4 log en el número de bacilos en la almohadilla plantar en relación a los controles no tratados.

RÉSUMÉ

L'activité anti-*Mycobacterium leprae in vivo* de la benzoxazinorifamycine, KRM-1648, nouvellement synthétisée, a été étudiée. Le KRM-1648 a été administré oralement à des souris nues athymiques, infectées par voie sous-cutanée dans la patte arrière par du *M. leprae*, à des doses entre 0,001 et 0,01 mg par souris et par jour six fois par semaine, du jour 31 au jour 80. L'administration de KRM-1648 supprimait de façon marquée la croissance bactérienne dans les coussinets plantaires pour 360 jours. Le KRM-1648 administré quotidiennement à la dose de 0,01 mg par souris entraînait une réduction de 2 à 4 log du nombre de bacilles acido-résistants. Les effets thérapeutiques du KRM-1648 étaient significativement plus élevés que ceux de la rifampicine quand les deux médicaments étaient donnés aux mêmes doses. De plus, quand les souris étaient nourries avec un régime contenant du KRM-1648 (0,00004%–0,0004%), le médicament montrait une efficacité encore plus élevée vis-à-vis de l'infection par *M. leprae*, causant une diminution de près de 4 log du nombre de bacilles lépreux dans les coussinets plantaires infectés par rapport aux témoins non traités.

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