Roxithromycin (RU 965): Effective Therapy for Experimental Syphilis Infection in Rabbits

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Roxithromycin (RU 965), a new macrolide antibiotic, was shown to be effective for therapy of active syphilis in rabbits. Dark-field-positive lesions were produced in adult male rabbits by intradermal inoculation of approximately 10^9 Treponema pallidum organisms at each of 11 sites. Beginning 7 days after infection, six animals per group were treated with benzathine penicillin G (200,000 U, intramuscularly, weekly for 2 weeks) or roxithromycin (15 mg/kg of body weight, orally, twice daily for 15 days); six animals were not treated. Chancres in untreated animals were dark-field positive throughout the 16-day observation period; all benzathine penicillin-treated rabbits were dark-field negative 1 day after the initiation of therapy. Five of six animals treated with roxithromycin were dark-field negative on day 3 following the initiation of therapy; the sixth animal was dark-field negative by day 6. Lesions in untreated animals reached a mean (± standard deviation) maximum diameter of 14.7 ± 1.91 mm compared with 8.4 ± 3.6 mm for benzathine penicillin-treated (P < 0.005) and 10.4 ± 1.2 mm for roxithromycin-treated (P < 0.001) animals. Ulceration occurred at 62 of 66 lesions in untreated animals compared with 0 of 66 lesions in each treated group. At 3, 6, and 12 weeks postinfection, Venereal Disease Research Laboratory antibody titers were significantly higher (P < 0.05) in untreated than in treated animals. Titors in penicillin-treated versus roxithromycin-treated animals were significantly different at 6 weeks postinfection but not at 3 and 12 weeks postinfection. Transfer of tissue from treated rabbits to seronegative recipient animals did not reveal any evidence of persistent infection in the benzathine penicillin- or roxithromycin-treated animals. These findings indicate that benzathine penicillin and roxithromycin, at the doses indicated above, are effective in treating active syphilis in the rabbit model.

Penicillin has long been the drug of choice for treatment of syphilis infection, and Treponema pallidum has shown no evidence of acquiring resistance to penicillin. For penicillin-allergic patients, however, the choice of alternative therapies is limited. The Centers for Disease Control (3) currently recommended tetracycline hydrochloride as alternative therapy, with erythromycin recommended for pregnant women and other tetracycline-intolerant individuals. Both tetracycline and erythromycin are given four times daily for 15 or 30 days, frequently resulting in the lack of patient compliance or drug intolerance. The development of a new antibiotic with a significantly prolonged half-life in serum might provide effective alternative therapy for syphilis, with better patient compliance and less gastrointestinal intolerance than experienced with more frequent doses.

Roxithromycin (RU 965; 9-[[2-(methoxyethoxy)ethoxy]imino]erythromycin) is a new macrolide antibiotic with a structure and spectrum of activity similar to those of erythromycin. It has several distinct advantages over erythromycin, however, including higher concentrations in plasma and tissue and a longer half-life (7 to 10 h in humans). For these reasons, it was anticipated that roxithromycin might be effective against T. pallidum with twice daily administration.

Experimental syphilis infection of rabbits provides an excellent model for evaluating antibiotic therapy against T. pallidum (1, 4, 5, 7, 9-11, 17). Intradermal inoculation results in the appearance of a dark-field-positive chancre which progressively ulcerates and then heals spontaneously. Persistent latent infection may be demonstrated in these animals by transfer of infective lymph node material to susceptible normal rabbits. In this report we describe the evaluation of roxithromycin as therapy for active syphilis in the rabbit model.

MATERIALS AND METHODS

Animals. Adult male New Zealand white rabbits were obtained from a local supplier and examined upon receipt for clinical or serological evidence of infection with Treponema paraluiscuniculi; infected animals were excluded from the study. All animals were fed antibiotic-free food and water, were housed at 18 to 20°C, and weighed 3.0 to 3.3 kg prior to infection.

T. pallidum. T. pallidum Nichols was originally obtained from James N. Miller (University of California, Los Angeles) and was maintained by serial passage in rabbits as described previously (12). For intradermal infection of rabbits, treponemes were extracted by gentle rotation of sliced infected testes in sterile 0.14 M saline with 10% heated (56°C, 30 min) normal rabbit serum. Gross tissue debris was removed from the resulting treponemal suspension by centrifugation for 7 min at 250 x g. The concentration of treponemes in the supernatant was determined by dark-field microscopy (13) and adjusted to 1.3 x 10^7 T. pallidum organisms per ml with 10% serum saline.

Infection of animals. Eighteen rabbits were divided randomly into three groups of six animals each. The clipped back of each animal was injected intradermally at each of 11 sites with 0.1 ml of treponemal suspension containing 1.3 x 10^7 virulent T. pallidum organisms per ml (1.3 x 10^6 T. pallidum organisms per skin site). The rabbits were clipped as necessary and examined daily for lesion development. When the syphilitic lesions reached a diameter of 8 to 10 mm (7 days postinfection), material was aspirated from one lesion on each animal and was examined by dark-field microscopy for evidence of motile T. pallidum.
TABLE 1. Therapeutic responses of syphilitic rabbits to roxithromycin or benzathine penicillin

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Therapy (total dose)</th>
<th>Maximum lesion diam (mm) (^a)</th>
<th>No. of ulcerative lesions/total</th>
<th>No. of animals with DF (^b)</th>
<th>Median time (days) to dark-field negativity</th>
<th>No. of infectious lymph nodes/total</th>
<th>Infection status posttherapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>None (control)</td>
<td>14.7 ± 1.9</td>
<td>62/66</td>
<td>6/6</td>
<td>&gt;15(^c)</td>
<td>1/1</td>
<td>Active</td>
</tr>
<tr>
<td>B</td>
<td>Benzathine penicillin (400,000 U)</td>
<td>8.4 ± 3.6</td>
<td>0/66</td>
<td>0/6</td>
<td>1</td>
<td>0/6</td>
<td>Adequately treated</td>
</tr>
<tr>
<td>C</td>
<td>1.5 g of roxithromycin (450 mg/kg)</td>
<td>10.4 ± 1.2</td>
<td>0/66</td>
<td>0/6</td>
<td>3</td>
<td>0/6</td>
<td>Adequately treated</td>
</tr>
</tbody>
</table>

\(^a\) Mean ± standard deviation.
\(^b\) DF\(^*\), Dark-field positive.
\(^c\) All rabbits were dark-field positive after 15 days.

Antimicrobial therapy. Antimicrobial therapy was initiated immediately following the first dark-field examination by the following schedule: group A, untreated controls; group B, benzathine penicillin G, 200,000 U per week, given intramuscularly at 1-week intervals for two doses; group C, roxithromycin, 15 mg/kg, given orally twice daily for 15 days. Roxithromycin was provided by Hoechst-Roussel Pharmaceuticals Inc. (Somerville, N.J.) and was packaged locally (50 mg per capsule) in no. 5 capsules using lactose filler. One 50-mg capsule represented a unit dose of approximately 15 mg/kg of body weight, and the total dose (1.5 g per rabbit) was equivalent (wt/wt) to the 30-g total dose recommended for erythromycin therapy of early syphilis in humans (3). The capsules were placed in the mouths of the animals until they were swallowed. Cages were checked 10 min later to ensure that the capsules had not been expelled. Rabbits were not fed within 3 h of drug administration. The benzathine penicillin G suspension for injection was purchased from Wyeth Laboratories (Philadelphia, Pa.).

Clinical and serological evaluation. For 15 days after the initiation of therapy, rabbit lesions were measured daily and examined for evidence of ulceration, healing, or both. On days 1, 2, 3, 6, 8, 10, 13, 14, and 15 of antimicrobial therapy and on the day following the completion of therapy, material was aspirated from one lesion on each rabbit and examined for the presence of motile \textit{T. pallidum} by dark-field microscopy. Lesions were determined to be dark-field positive if a motile treponeme was observed. Lesions were declared dark-field negative if no motile treponemes were seen during a 7-min period (approximately 100 microscope fields). After three successive dark-field-negative examinations, a rabbit was not examined further by this method. The Venereal Disease Research Laboratory (VDRL; Scientific Products, Inc., McGaw Park, Ill.) serum titers and fluorescent treponemal antibody-absorbed (FTA-ABS; Palomar Chemicals, Carlsbad, Calif.) reactivities of all animals were determined immediately prior to commencement of antimicrobial therapy and at regular intervals thereafter until 12 weeks following infection.

Determination of latent infection following therapy. Animals that showed no clinical evidence of persistent or recurrent infection (all group B and C rabbits) were sacrificed at the end of the observation period and evaluated for latent infection by tissue transfer (13). Popliteal lymph nodes from each animal were removed and forced through a stainless steel screen into 2 to 4 ml of sterile saline containing 50% normal rabbit serum; the extracted material was examined by dark-field microscopy for the presence of \textit{T. pallidum} and was injected intratesticularly into serologically nonreactive recipient rabbits. The recipient rabbits were examined regularly for clinical (development of dark-field-positive orchitis) or serological (seroconversion in VDRL and FTA-ABS tests) evidence of syphilis infection. If, at the end of the 3-month observation period, a recipient animal showed no evidence of infection, the transferred lymph node material was judged to be noninfectious, and the donor animal was considered to have been adequately treated.

Serologic tests. The VDRL slide flocculation (Scientific Products) and FTA-ABS (Palomar) tests were performed as described previously (14), with known negative and positive rabbit serum samples used as controls. The FTA-ABS test was modified for rabbit serum by using fluorescein-labeled goat anti-rabbit immunoglobulin G (Cappel Laboratories, West Chester, Pa.) at a final dilution of 1/3,200.

Statistical analysis. VDRL titers for treatment groups were compared by the Mann-Whitney U test. Maximum lesion sizes were compared among treatment groups by analysis of variance with repeated measurements. The time required to achieve dark-field negativity was compared among treatment groups by the Mantel-Cox chi-square test. All \(P\) values were based on two-tailed tests.

RESULTS

Clinical evaluation of dermal lesions. Untreated control rabbits (group A) developed large ulcerative lesions during the 15-day observation period, reaching a mean maximum diameter of 14.7 ± 1.9 mm (Table 1). Of 66 lesions, 62 (94%) were ulcerated. Motile organisms were readily demonstrable in lesion aspirate material throughout the observation period. On the other hand, treatment of infected rabbits with benzathine penicillin (group B) resulted in the rapid elimination of motile organisms from dermal lesions. No viable \textit{T. pallidum} could be demonstrated in any of the six rabbits 24 h following benzathine penicillin administration (\(P < 0.001\), compared with the untreated controls). Lesions in the benzathine penicillin treated rabbits did not ulcerate (0 of 66), and lesions were resolved by the end of the observation period. The maximum lesion diameter achieved in this group was 8.4 ± 3.6 mm (\(P < 0.005\), compared with untreated controls).

Rabbits that were treated with roxithromycin also demonstrated rapid elimination of \textit{T. pallidum} from lesions (\(P < 0.001\), compared with untreated controls) and healing without ulceration (0 of 66 lesions in untreated controls). Five of six animals were dark-field negative 3 days after the initiation of therapy; the sixth rabbit was dark-field negative on day 6. The mean maximum lesion diameter achieved in this group was 10.4 ± 1.2 mm (\(P < 0.001\), compared with untreated controls). The median time to dark-field negativity in the roxithromycin-treated animals, however, was longer than that in the benzathine penicillin-treated group (3 versus 1 day; \(P = 0.005\)).

Serologic studies. Serologic testing was performed immediately prior to the initiation of therapy and at 3, 6, and 12
weeks postinfection. All animals were reactive by the FTA-ABS test prior to the initiation of therapy (day 7 following infection) and remained reactive thereafter, regardless of therapy.

Prior to the initiation of therapy, VDRL titers were not significantly different among the three groups. However, at 3, 6, and 12 weeks postinfection the mean titers for the benzathine penicillin- and roxithromycin-treated groups were significantly lower than that for the untreated group \( (P < 0.04) \). The roxithromycin-treated animals differed significantly from the benzathine penicillin-treated animals only at 6 weeks postinfection \( (P = 0.004) \); at 3 and 12 weeks, titers were not significantly different between these two groups.

**Determination of latent infection.** Animals that had demonstrable motile *T. pallidum* at the end of therapy (in this study, only the untreated animals) were still infected. All other rabbits (benzathine penicillin- and roxithromycin-treated) were asymptomatic and were evaluated for the presence of persistent *T. pallidum* infection by papillary lymph node transfer to serologically nonreactive recipient rabbits. One untreated rabbit was included as a positive control. No clinical or serologic evidence of infection was detectable during a 3-month observation period in the recipients of tissue from the benzathine penicillin- or roxithromycin-treated rabbits. The donor animals were thus considered to have been adequately treated, with no persistent latent infection. The recipient of the tissue from the untreated rabbit became serologically reactive 1 month following tissue transfer, and material aspirated from the testes was dark-field positive.

**DISCUSSION**

Erythromycin has been recommended as an alternative drug for the treatment of syphilis in penicillin-allergic patients. Limited efficacy studies of various erythromycin preparations (mostly estolate) have shown that total doses lower than 30 g (approximately 400 mg/kg) result in high treatment failure rates in patients with early syphilis. Total doses of 10 to 20 g yielded cumulative 1-year retreatment (failure and reinfection) rates of 12 to 30% (for reviews, see references 2 and 6). In a study of erythromycin base done by Schroeder et al. (15), the treatment failure rates following 20- and 30-g total doses were 17.5 and 4%, respectively, with cumulative retreatment rates of 26.8 and 10.9%, respectively. The efficacy of erythromycin for therapy of syphilis in penicillin-allergic pregnant women has also been questioned (8, 16), but its effectiveness in neurosyphilis has not been examined. Because of its short half-life in serum (1.4 h), erythromycin is administered four times daily for 15 or 30 days (3) to maintain consistent levels for activity against *T. pallidum*. The inconvenience of dosing four times daily frequently results in poor patient compliance, which may contribute to the failure rate.

With these considerations in mind, roxithromycin was evaluated in the treatment of active syphilis infection in the rabbit model. Efficacy was assessed by the rate of clearance of *T. pallidum* from dermal lesions, resolution of the lesions, and the presence or absence of infectious organisms in transferred lymph node material following therapy. Roxithromycin was effective in treating syphilis in this model when used at a dosage of 50 mg (approximately 15 mg/kg) orally, twice daily for 15 days. Bacteria were cleared from the lesions of roxithromycin-treated animals, although more slowly than from those of the penicillin-treated animals, and no evidence of persistent infection was found by lymph node transfer.

The comparatively poor pharmacokinetic characteristics of roxithromycin in rabbits (half-life, < 30 min) compared with those in humans (half-life, 7 to 10 h) make the results of this study even more impressive with regard to the potential usefulness of roxithromycin for humans. The dose of roxithromycin used in this study was equivalent (wt/wt) to the 30-g total dose recommended for erythromycin therapy in humans; lower doses were not examined because of the poor efficacy of lower doses of erythromycin in humans. Toxicity studies of roxithromycin in humans indicate that 600 mg per day for 10 days is well tolerated; higher doses have not been examined. Rats and dogs tolerate doses of 100 to 200 mg/kg per day for 14 days, which is equivalent (wt/wt) to a total human dose of 100 to 200 g. Because higher levels in serum and tissue are achieved with roxithromycin than with erythromycin, effective therapy for syphilis in humans could probably be achieved with lower doses or less frequent administration than those used in rabbits. Pharmacokinetic studies in humans have shown that the half-life of roxithromycin increases to approximately 17 h with repeated drug administration, and it may be possible to treat patients once a day.

If roxithromycin were to be considered as an alternative to erythromycin in the therapy of syphilis, penetration of the drug across the blood-brain barrier and the placenta would be an important consideration. Recommended alternative therapies for penicillin-allergic pregnant patients or for patients with central nervous system involvement have not been demonstrated to be highly efficacious, and it is particularly for these patients that new effective therapies should be examined. The demonstrated efficacy of roxithromycin against *T. pallidum* in the rabbit model and its superior pharmacokinetics in humans make it a good candidate for further evaluation.

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**LITERATURE CITED**


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