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Efficacy of Cefmetazole in the Treatment of Active Syphilis in the Rabbit Model

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Cefmetazole, a cephamycin-type antibiotic, was shown to be as effective as standard benzathine penicillin for therapy of active syphilis in the rabbit model. Four groups of six adult male rabbits were inoculated intradermally with 10^6 *Treponema pallidum* per site, producing primary syphilitic lesions. One week following infection, groups of rabbits were treated with benzathine penicillin (200,000 U intramuscularly weekly for 2 weeks) or cefmetazole (20 or 40 mg/kg per day intramuscularly in four divided doses for 15 days); one group was untreated. Daily dark-field microscopic examination of lesion aspirates demonstrated that the mean times to dark-field negativity were the same for benzathine penicillin- and two cefmetazole-treated groups (1.0. 1.0, and 1.17 days, respectively), while all untreated animals remained dark-field positive for >15 days. Mean maximum lesion diameters in cefmetazole-treated animals (8.7 ± 1.3 and 8.1 ± 1.3 mm) were equivalent to those in penicillin-treated animals (8.6 ± 1.6 mm) and were smaller than observed in untreated animals (12.4 ± 2.2 mm; $P < 0.01$); fewer lesions ulcerated in penicillin- or cefmetazole-treated rabbits than in untreated rabbits ($P < 0.001$). Persistent infection was documented in lymph nodes of untreated rabbits; no evidence of latent infection was found in penicillin- or cefmetazole-treated animals.

Penicillin remains the drug of choice for treatment of all forms of syphilis, providing safe and effective therapy for most individuals. For those persons who are allergic to penicillin, however, alternative therapies are limited. The currently recommended alternative therapies are tetracycline and, for patients who cannot tolerate tetracycline, erythromycin (4). Both regimens require dosing four times daily, and patient compliance may be poor. Further, the efficacy of these alternative therapies in patients with central nervous system (CNS) involvement is unknown. It is therefore important to identify other alternative antibiotics with treponemicidal capabilities, particularly in light of the increasing reports of treatment failure in syphilis patients concurrently infected with the human immunodeficiency virus following standard benzathine penicillin therapy (2, 21).

Cefmetazole is a parenterally administered, cephamycin-type, β-lactamase-resistant antibiotic. It is active against a wide range of aerobic and anaerobic gram-positive and gram-negative bacteria, including members of the family *Enterobacteriaceae*, nonenterococcal streptococci, methicillin-susceptible *Staphylococcus* spp., *Branhamella catarrhalis*, *Haemophilus influenzae*, and *Neisseria* spp. (15, 24, 26). It was anticipated that this agent might also be effective against *Treponema pallidum*, and studies were initiated in the rabbit model.

Intradermal infection of the rabbit with *T. pallidum* closely resembles the primary disease seen in natural infection in humans and provides an excellent model for evaluation of the efficacy of new antibiotic regimens (1, 5, 6, 9, 14, 17–19). Dark-field (DF)-positive lesions appear at the site of inoculation, progressively ulcerate, and heal spontaneously, following a predictable time course. Latent infection can be confirmed by transferring infected lymph node material to normal rabbits and documenting seroconversion. This report evaluates the efficacy of cefmetazole for treatment of active syphilis by using this experimental model.

MATERIALS AND METHODS

Animals. Adult male New Zealand White rabbits (2.5 to 3.0 kg) were obtained from R & R Rabbitry, Stanwood, Wash., and examined upon receipt for clinical or serological evidence of infection with *Treponema paraluiscuniculi*. Only rabbits free of this infection were included in this study. Rabbits were fed antibiotic-free food and water and were housed at 18 to 20°C.

T. pallidum. *T. pallidum* subsp. *pallidum* (Nichols strain) was maintained by testicular passage in rabbits (20), and treponemes were prepared for intradermal inoculation of rabbits as described previously (18).

Intradermal infection. Twenty-four rabbits were randomly divided into four groups of six animals each. The shaved back of each rabbit was injected intradermally with 0.1 ml of treponemal suspension (10^7 virulent *T. pallidum* per ml) at each of 10 sites (10^6 per site). The rabbits were shaved 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 examined by DF microscopy for the presence of motile *T. pallidum*.

Serological tests. The Venereal Disease Research Laboratory (VDRL) slide flocculation (American Scientific Products, McGaw Park, Ill.) and fluorescent treponemal antibody-absorbed (FTA-ABS) tests (Zeuss Scientific, Inc., Raritan, N.J.) were performed as described in the *Manual of Tests for Syphilis* (27). Known reactive and nonreactive rabbit sera were used as controls. The FTA-ABS test was modified for rabbit serum with fluorescein isothyocyanate-labeled goat anti-rabbit immunoglobulin G (Organon Teknika–Cappel, West Chester, Pa.) at a final dilution of 1/3,200.

Antimicrobial therapy. Antimicrobial therapy was initiated immediately following the first DF examination described

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TABLE 1. Weight gain during therapy[a]

Drug (total dose)	Mean wt gain ± SD (kg)
None (control)	0.40 ± 0.08
Benzathine penicillin G (400,000 U)[b]	0.43 ± 0.17
Cefmetazole (0.9 g)	0.21 ± 0.18[c]
Cefmetazole (1.8 g)	0.26 ± 0.13

[a] Rabbits which died were omitted from analysis.
[b] One milligram of penicillin G equals 1,666 U.
[c] Statistically significant when compared with control group (analysis of variance; $P = 0.03$).

above. Cefmetazole, supplied by The Upjohn Co., Kalamazoo, Mich., was suspended in sterile water and stored at 4°C until use (within 24 h). Two different dosages of cefmetazole were each administered to separate groups of rabbits four times daily intramuscularly for 15 days. The daily doses of 20 (total dose, 0.9 g) and 40 (total dose, 1.8 g) mg/kg in rabbits are roughly equivalent (per kilogram) to human doses of 1.5 and 3.0 g/day. Penicillin G benzathine suspension for injection was purchased from Wyeth Laboratories, Philadelphia, Pa.; rabbits received 200,000 U given intramuscularly twice, 1 week apart. One group remained untreated.

Clinical and serological evaluation. Daily, for 15 days after initiation of therapy, rabbit lesions were measured and examined for evidence of ulceration or healing and aspirated material from one lesion on each rabbit was examined for the presence of motile *T. pallidum* by DF microscopy. Lesions were determined to be DF negative if no motile treponemes were seen during a 7-min period (approximately 100 microscope fields). After three successive DF-negative examinations, a rabbit was not examined further by this method. Serum VDRL titers and FTA-ABS reactivities of all animals were determined immediately prior to commencement of antimicrobial therapy (1 week postinfection) and at 3 and 6 weeks postinfection.

Determination of latent infection following therapy. Animals which were DF negative after completion of the treatment regimen and showed no clinical evidence of persistent or recurrent infection following therapy were sacrificed (6 weeks postinfection) and evaluated for latent infection by tissue transfer (22). Popliteal lymph nodes from each animal were removed and forced through a stainless-steel mesh into 2 to 4 ml of 50% serum saline. The extracted material was examined by DF microscopy for the presence of *T. pallidum* and was injected intratesticularly into serologically nonreactive recipient rabbits. The recipient rabbits were examined regularly for clinical (development of DF-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 noninfectious and the donor animal was considered to have been adequately treated. Lymph nodes from one animal from the untreated group were also passaged as a positive control for this procedure.

Statistical analysis. Geometric mean VDRL titers, mean maximum lesion sizes, proportion of ulcerated lesions, and weight change during therapy were compared between treatment groups by analysis of variance with repeated measures. Median time required to achieve DF negativity was compared between treatment groups by Mantel-Cox chi square. All P values are based on two-tailed tests.

RESULTS

Tolerance of drug therapy. Two animals (one in the untreated group and one in the higher-dose cefmetazole group) died during the observation period. The deaths could not be directly attributed to drug therapy or other procedures, although the rabbit in the cefmetazole group suffered diarrhea prior to death. Cefmetazole-treated rabbits gained less weight during the observation period than penicillin-treated or untreated rabbits (Table 1); this finding was statistically significant, however, only for rabbits receiving the lower dose of cefmetazole. It could not be determined whether the drug was directly responsible for these differences or whether discomfort due to frequent intramuscular injections contributed to a loss of appetite. No other adverse side effects, such as diarrhea or skin eruptions, were evident in any of the surviving rabbits.

Clinical evaluation of dermal lesions. Untreated control rabbits developed large, ulcerative lesions during the 15-day observation period (Table 2); lesions reached a mean maximum diameter of 12.4 ± 2.2 mm. Of 50 lesions, 43 (88%) ulcerated. Motile organisms were readily demonstrable in lesion aspirate material from all untreated animals throughout the observation period. Treatment of infected rabbits with benzathine penicillin resulted in the rapid elimination of motile organisms from dermal lesions; no viable *T. pallidum* could be demonstrated in any of these six rabbits after the first day of therapy ($P = 0.0016$, compared with untreated). Lesions in the penicillin-treated rabbits ulcerated less frequently (8 of 56; 14%) and resolved by the end of the observation period. The maximum lesion diameter achieved in this group was 8.6 ± 1.6 mm ($P < 0.001$, compared with untreated).

The mean maximum diameters for lesions in the rabbits which received higher and lower doses of cefmetazole (8.7 ± 1.3 and 8.1 ± 1.3 mm, respectively) were similar to those

TABLE 2. Therapeutic response of syphilitic rabbits to cefmetazole or benzathine penicillin

Therapy (total dose)	No. of rabbits in group	Maximum lesion diam ± SD (mm)	No. of ulcerative lesions/total	Mean time (range) to DF negativity (days)	No. of rabbits with DF-positive lesions at completion of therapy/total
None (control)	6[a]	12.4 ± 2.2	43/50[b]	>15[c]	5/5
Benzathine penicillin G (400,000 U)	6	8.6 ± 1.6[d]	8/56	<1 (1)	0/6
Cefmetazole (0.9 g)	6	8.7 ± 1.3[d]	0/59	<1 (1)	0/6
Cefmetazole (1.8 g)	6[a]	8.1 ± 1.3[d]	0/48[b]	<1 (1–2)	0/6[e]

[a] One rabbit died in group prior to completion of therapy.
[b] Data for five rabbits completing therapy.
[c] All rabbits in group were still DF positive after 15 days.
[d] Statistically significant compared with control group.
[e] The rabbit that died was DF negative prior to death.

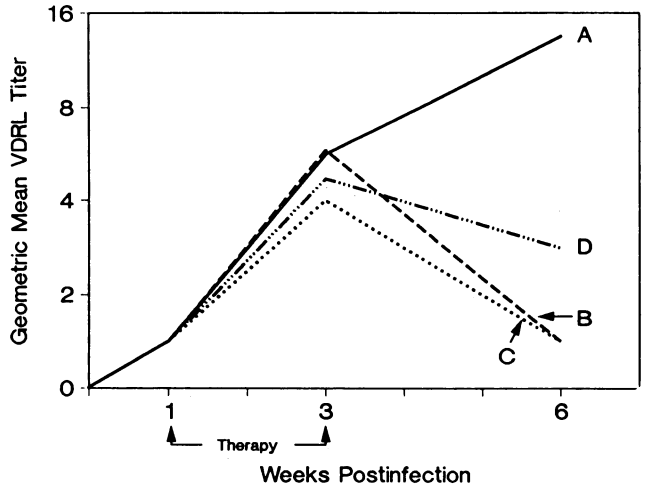

FIG. 1. Geometric mean VDRL titers of rabbits receiving no (A), benzathine penicillin (B), or cefmetazole (0.9 or 1.8 g; C and D, respectively) therapy. Significant differences between untreated and all treated groups of rabbits were noted only at 6 weeks postinfection.

seen in the penicillin-treated rabbits ($P = 0.69$ and 0.57, respectively, compared with benzathine penicillin). The lesions failed to ulcerate and were significantly ($P < 0.0001$) smaller than in the untreated animals. All but one of the cefmetazole-treated rabbits (high-dose group) became DF negative within 1 day following initiation of therapy; the remaining rabbit was DF negative by the second day.

Serological studies. Serological testing was performed immediately prior to initiation of therapy and at 3 and 6 weeks postinfection (Fig. 1). Prior to initiation of therapy and 3 weeks postinfection, VDRL titers were not significantly different between groups. At 6 weeks postinfection, however, all treated rabbits had significantly lower titers than the untreated controls ($P < 0.0001$). No significant differences were evident in posttreatment titers among the three groups of treated animals.

Determination of latent infection. Untreated rabbits still had demonstrable motile *T. pallidum* in lesion exudate at the end of therapy and were obviously still infected. All other rabbits (benzathine penicillin and cefmetazole treated) were asymptomatic and were evaluated for the presence of persistent *T. pallidum* by popliteal lymph node transfer to serologically nonreactive recipient rabbits. One untreated rabbit was included as a positive control. No clinical or serological evidence of infection was observed during a 3-month period in any of the recipients of tissue from the benzathine penicillin- or cefmetazole-treated rabbits (Table 3). The donor animals were thus all considered to have been adequately treated, with no persistent latent infection. As expected, the recipient of the tissue from the untreated rabbit became serologically reactive 1 month following tissue transfer, and material aspirated from the testes was DF positive.

TABLE 3. Evaluation of persistent latent infection in treated rabbits[a]

Therapy (total dose)	No. of recipients positive for[b]:		Status of infection in donor group
	T. pallidum	Reactive VDRL + FTA-ABS	
None (control)[c]	1/1	1/1	Infected
Benzathine penicillin G (400,000 U)	0/6	0/6	Adequately treated
Cefmetazole (0.9 g)	0/6	0/6	Adequately treated
Cefmetazole (1.8 g)	0/5	0/5	Adequately treated

[a] Popliteal lymph nodes were excised from rabbits 6 weeks postinfection, minced, and inoculated intratesticularly into normal recipient animals.
[b] Number positive/total number evaluated.
[c] Only one representative rabbit from the untreated group was evaluated as a control.

DISCUSSION

After decades of use, penicillin remains the drug of choice for therapy for all stages of syphilis (4). To date, there have been no reports documenting the existence of penicillin-resistant strains of *T. pallidum*. However, alternative antibiotic therapy is required for individuals who are allergic to penicillin. Currently recommended alternatives, tetracycline and erythromycin (4), have several disadvantages: (i) patient compliance may be poor because of required four-times-daily dosing for periods of 2 to 4 weeks; (ii) both tetracycline (10) and erythromycin (8) exhibit poor CNS penetration and their therapeutic efficacy in inapparent or known neurological involvement has not been closely examined; and (iii) neither drug is appropriate for treatment of syphilis in pregnant women. Furthermore, the treatment failure rate is higher in patients treated with erythromycin (3, 7, 28) and an erythromycin-resistant strain of *T. pallidum* has been isolated (30). For these reasons, new alternatives to penicillin should be sought.

Some other broad-spectrum cephalosporins have been evaluated for ability to immobilize *T. pallidum* in vitro; both ceftriaxone and ceftizoxime were found to be as effective as penicillin G at two- to fivefold-higher concentrations (16). Cefoperozone (and others) has not been examined, however. Ceftriaxone has been evaluated in the rabbit model (14) and was shown to be very effective in the treatment of early experimental syphilis. Clinical studies and case reports have also shown ceftriaxone to be useful in the treatment of incubating and early syphilis (12, 13, 23) and asymptomatic neurosyphilis (12).

Both of the dosages of cefmetazole examined in this study were completely effective for treatment of active syphilis in the rabbit model, and the period of presumed infectivity following initiation of therapy (time to DF negativity) was as short as with penicillin treatment. We did not examine the efficacy of cefmetazole given twice daily or for a shorter duration. Despite its efficacy, however, the requirement for intravenous or intramuscular administration and the relatively short half-life of cefmetazole make it impractical for routine syphilis therapy. Recent studies (29, 31) have shown that colonic absorption of this antibiotic can be increased under experimental conditions by the addition of promotors, such as sodium EDTA, suggesting that oral administration may become possible in the future.

Two major areas of continuing discussion concerning syphilis therapy are treatment for patients with neurosyphilis and penicillin-allergic pregnant women. It is for these clinical settings that new alternatives should be sought. Little is known about penetration of cefmetazole into the placenta or the CNS. Intravenous administration of 1 g of cefmetazole to women prior to delivery revealed that near-peak levels in umbilical cord blood (approximately 12 μg/ml) were reached 1 h following injection and persisted through 2.5 h (33).

These levels were about 12% of peak levels in serum (0.5 h postinjection). The peak level in amniotic fluid was only 4 μg/ml (approximately 4% of levels in serum) at 3 h; no information is available concerning fetal tissue levels. Minimum treponemicidal levels have not yet been established for this drug.

Penetration of cefmetazole into the CNS in humans has been less well defined. Two cases of neonatal meningitis caused by *Escherichia coli* were treated with 50 mg/kg four times daily for 15 days or 90 mg/kg three times daily for 12 days, and complete cure was obtained (11). Drug levels in the cerebrospinal fluid of one patient given a single dose of 50 mg/kg were 20.3 and 34.5 μg/ml at 0.5 and 1 h, respectively. One case of neonatal meningitis caused by a *Flavobacterium* sp. was also successfully treated with cefmetazole (32). Clearly, no conclusion about the effectiveness of cefmetazole for treatment of neurosyphilis can be drawn until more extensive information regarding the pharmacokinetics of CNS penetration and minimum drug levels required for *T. pallidum* susceptibility have been determined. An in vitro system for determining antimicrobial susceptibility of *T. pallidum* has been proposed (16, 25) and minimum treponemicidal levels of cefmetazole could be established.

Finally, because multiple sexually transmitted diseases are frequently acquired simultaneously, it is important to consider the efficacy of cefmetazole in treatment of such diseases. *Neisseria gonorrhoeae* has been shown to be susceptible to low levels of cefmetazole in vitro (15); limited clinical trials with 1 g of cefmetazole plus 1 g of oral probenecid, however, did not achieve bacteriological cure in all patients (H. H. Handsfield, personal communication). The results of the study described here suggest that treatment of gonorrhea with cefmetazole should be successful in patients recently exposed to (incubating) syphilis.

Our results indicate that cefmetazole has substantial activity against *T. pallidum* and, at the doses tested, is as effective as benzathine penicillin. Although its relatively short half-life and current requirement for parenteral administration are practical disadvantages to its use in syphilis therapy, it should be further examined and might prove valuable as an alternative therapy for some penicillin-allergic patients, particularly if good penetration into the CNS and fetal tissue is demonstrated.

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