Comparison of the Activities of Ceftriaxone and Penicillin G Against Experimentally Induced Syphilis in Rabbits

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The activity of ceftriaxone, a newly developed cephalosporin, against early cutaneous infections with Treponema pallidum in rabbits was compared with that of equimolar doses of penicillin G. Activity was related to the time required for cutaneous lesions to become dark-field negative, serological response, and the disappearance of T. pallidum from the popliteal lymph nodes. Both antibiotics were very effective in the treatment of syphilis in this animal model. The 50% curative dose for penicillin G was 0.8 μmol/kg (0.29 mg or 480 U/kg) and for ceftriaxone, it was 1.45 μmol/kg (0.96 mg/kg). Overall, ceftriaxone was slightly less effective than penicillin G was. Transmission and scanning electron microscopy studies of testicular aspirates obtained from rabbits treated with ceftriaxone revealed alterations in the treponeme surface which apparently resulted in changes in cell permeability and morphology.

Ceftriaxone is a newly developed parenteral cephalosporin. It has a broader spectrum of activity, higher potency, and better β-lactamase stability than the older semisynthetic cephalosporins; it is also cleared from the body more slowly, with a plasma half-life of approximately 8 h in humans (9). Ceftriaxone penetrates into the cerebrospinal or interstitial fluid readily and is eliminated from these compartments more slowly than related β-lactam antibiotics (8).

Preliminary studies with ceftriaxone suggest that it may be effective as a one-dose treatment for uncomplicated acute gonorrhea (4). As dual infections with syphilis and gonorrhea do occur, the antitreponemal activity of this antibiotic needs to be determined. This report describes the evaluation of ceftriaxone for its effectiveness in the treatment of experimentally induced syphilis in rabbits. Penicillin G was included in this study for comparison purposes.

MATERIALS AND METHODS

Antibiotics. Ceftriaxone, (6R,7R)-7-[2-amino-4-thiazolyl]-2-methoxyimino)acetamido]-3-[(2,5-dihydroxy-6-hydroxy-2-methyl-5-oxo-3-triazin-3-yl)thio][methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (disodium salt), was obtained from Hoffmann-La Roche Inc., Nutley, N.J. Penicillin G sodium for injection was purchased from E. R. Squibb & Sons, Inc., Princeton, N.J.

Serological test. The Rapid Plasma Reagin (RPR) card test (Hynson, Wescott and Dunning, Baltimore, Md.) was used to follow the development of antilipid antibodies resulting from infection with Treponema pallidum. The antigen used in the RPR test is a carbon-containing cardiolipin.

Animals. Altogether, 110 healthy male Dutch Belt rabbits weighing 2 to 3 kg and responding negatively to the RPR test were used for intratesticular passage of treponemes and the drug evaluation studies. They were housed at 19 to 21°C and provided antibiotic-free food and water ad libitum.

T. pallidum. The Nichols strain of T. pallidum was used exclusively in this study. It was maintained by intratesticular passages with approximately 107 treponemes per inoculum. After the development of orchitis, usually by 9 to 14 days, rabbits were killed by intracardiac injection of sodium pentobarbital. The testes were removed, rinsed in physiological saline, placed in saline containing 50% rabbit serum (pH 7.2), sliced, extracted aerobically for 20 to 30 min at 28°C, and then centrifuged at 1,000 × g for 7 min at 28°C. The supernatant was decanted and centrifuged at 20,000 × g for 30 min to concentrate the organisms, which were then suspended in 2 to 3 ml of Eagle minimal essential medium. The number of treponemal cells in this latter suspension was counted in a Petroff-Haussner chamber.

Challenge and drug evaluation procedures. The experimental procedures used were similar to those described by Clark and Yobs (3). The shaved backs of rabbits were injected intracutaneously with 107 or more cells of T. pallidum at each of six sites. The inoculated animals were randomly divided into treatment groups of five rabbits each, with nine animals serving as untreated controls. Treatment was initiated after 7 to 10 days, when cutaneous lesions were dark-field positive and measured 7 to 12 mm in diameter. The treatment schedule consisted of one daily intramuscular injection of aqueous solutions of sodium salts of the test drugs for 5 days, resulting in total dosages of 5, 2.5, 1.25, and 0.625 μmol/kg.

Cutaneous lesions were examined daily for regression in size and the presence of T. pallidum. Lesions were considered negative for treponemes when dark-
TABLE 1. Therapeutic evaluation of rabbits with experimentally induced syphilis treated with ceftriaxone or penicillin G

| Drug       | Total dose | No. of rabbits | Mean days | Mean days | No. of rabbits |
|------------|------------|----------------|-----------|-----------|--------------|
|            | µmol/kg    | mg/kg in group | to negative | to healed | developing |
| Ceftriaxone|            |                | smears    | primary  | secondary   |
| 5          | 3.3        | 4              | 1.0       | 8.0       | 0            |
| 2.5        | 1.65       | 5              | 1.2       | 10.8      | 0            |
| 1.25       | 0.825      | 5              | 1.6       | 8.4       | 2            |
| 0.625      | 0.413      | 5              | 1.6       | 10.0      | 5            |
| Penicillin G*| 5          | 1.8            | 1.2       | 8.2       | 0            |
| 2.5        | 0.9        | 4              | 1.25      | 7.5       | 0            |
| 1.25       | 0.45       | 5              | 1.8       | 11.5      | 2            |
| 0.625      | 0.225      | 5              | 1.8       | 7.6       | 3            |
| Control    | 9          | 43             | 51.5      |           | 9            |

* One milligram of penicillin G equals 1,666 U.

field examination of lesion exudates failed to reveal their presence on three successive days. The serological response of the animals was monitored by the RPR card test when treatment was initiated and at 6 months posttreatment. At 6 months after treatment, popliteal lymph nodes from treated and untreated rabbits were excised and minced in 2 ml of Eagle minimal essential medium. After 15 to 20 min, 1 ml of the supernatant was injected into the testis of a normal rabbit. At 2 months after this injection, RPR antibody responses were measured, and aspirates were obtained from testes exhibiting orchitis and examined for the presence of T. pallidum.

This study was initiated with some animals receiving drug doses of 5 and 2.5 µmol/kg and four control rabbits. After 2 months, other rabbits were treated with 1.25 or 0.625 µmol of the antibiotics per kg and five additional control animals were introduced into the investigation. This schedule was followed to facilitate the numerous observations necessary while cutaneous lesions were healing.

Calculation of CD50. The 50% curative doses (CD50) for ceftriaxone and penicillin were calculated by the method of Litchfield and Wilcoxon (7).

Electron microscopy. Each of six rabbits was inoculated intratesticularly with 10^7 T. pallidum cells per testis. After the development of orchitis, three rabbits were given single doses of 5 µmol of ceftriaxone per kg intramuscularly, and the remaining three served as untreated controls. Testicular aspirates were obtained hourly for 6 h after dosage and examined by dark-field and electron microscopy. Specimens for transmission electron microscopy were prepared by the procedure of Kellenberger et al. (6) and those for scanning electron microscopy were prepared by the method of Carleton et al. (2).

RESULTS

T. pallidum was rapidly eliminated from the cutaneous lesions of rabbits treated with either ceftriaxone or penicillin G (Table 1). The mean time required for lesions of the ceftriaxone-treated rabbits to become dark-field negative was 1.0 day for recipients of the 5-µmol/kg dose and 1.6 days for recipients of the 0.625-µmol/kg dose. The corresponding values for rabbits treated with the same doses of penicillin G were 1.2 and 1.8 days, respectively. In contrast, T. pallidum persisted for an average of 43 days in the lesions of untreated rabbits.

The primary lesions of rabbits treated with either ceftriaxone or penicillin healed within 7.5 to 11.5 days after initiation of therapy (Table 1). The lesions of untreated rabbits required an average of 51.5 days to heal. Secondary lesions did not develop in rabbits treated with 5 or 2.5 µmol of ceftriaxone or penicillin per kg (Table 1). Two of five recipients of ceftriaxone at the 1.25-µmol/kg dose and all five recipients of the 0.625-µmol/kg dose developed secondary lesions. Secondary lesions appeared in two of five and three of five recipients of penicillin in doses of 1.25 and 0.625 µmol/kg, respectively (Table 1). All of the untreated rabbits developed secondary lesions.

The antibody response to infection by T. pallidum in treated and untreated rabbits was monitored by the RPR card test. All of the rabbits became RPR positive, with titers ranging from 1:2 to 1:16, at the time of initiation of treatment. As shown in Table 2, 12 of the 18 surviving ceftriaxone-treated rabbits were RPR positive at 6 months posttreatment. Only 4 of the 15 penicillin-treated rabbits were RPR positive at that time. The largest number of RPR-positive rabbits and the highest RPR titers were found in the recipients of the lowest doses of both drugs. All of the untreated control rabbits were RPR positive at the 6-month target date.

The results of the examination of popliteal nodes of treated and untreated rabbits at 6 months posttreatment are summarized in Table 2. T. pallidum was not detected in recipients of
TABLE 2. Evaluation of popliteal lymph nodes

| Drug        | Dose (μmol/kg) | No. of donors | No. RPR positive¢ | No. of recipients positive for: |
|-------------|---------------|---------------|-------------------|---------------------------------|
|             |               |               |                   | T. pallidum | RPR‡ |
| Ceftriaxone | 5             | 3             | 2 (2)             | 0 | 0 |
|             | 2.5           | 5             | 2 (±2)            | 1 | 1 (64) |
|             | 1.25          | 5             | 3 (2–8)           | 2 | 2 (8–64) |
|             | 0.625         | 5             | 5 (8–32)          | 4 | 5 (4–64) |
| Penicillin G| 5             | 4             | 1 (2)             | 0 | 0 |
|             | 2.5           | 4             | 0                 | 0 | 0 |
|             | 1.25          | 4             | 1 (8)             | 1 | 1 (64) |
|             | 0.625         | 3             | 2 (8–16)          | 2 | 2 (32–64) |
| Control     |               | 9             | 9 (±64)           | 7 | 9 (2–64) |

¢ Popliteal lymph nodes were excised from treated and untreated rabbits 6 months after initial treatment. Lymph node preparations were then injected into the testes of normal rabbits; after 2 months, antibody responses were measured by the RPR card test, and aspirates obtained from testses exhibiting orchitis were examined for the presence of T. pallidum.

‡ Measured by RPR card test 6 months after treatment.

The reciprocal of the RPR titer or the titer range is given in parentheses.

Lymph node extracts from any rabbit treated with 5 μmol of ceftriaxone or penicillin per kg. Spirochetes were found in one of five, two of three, and four of five recipients of lymph node extracts from animals treated with 2.5, 1.25, and 0.625 μmol of ceftriaxone, respectively. The corresponding results for recipients of lymph node extracts from rabbits receiving the same doses of penicillin were zero of four, one of four, and two of three, respectively. Seven of nine recipients of untreated rabbit lymph node extracts were positive for T. pallidum. All recipient rabbits with positive testicular aspirates became RPR positive. Although one recipient of lymph node extract from rabbits treated with 0.625 μmol of ceftriaxone per kg and two recipients of untreated rabbit lymph node extracts became RPR positive, T. pallidum was not detected in these animals.

Results of the lymph node extract study showed that the serological test provided a more sensitive indicator of infection than dark-field

FIG. 1. Transmission electron micrograph of negatively stained T. pallidum (Nichols strain) from a rabbit 4 h after treatment with 5 μmol of ceftriaxone per kg. Note the granular appearance of the cell body, indicating penetration of PTA. Bar, 0.5 μm.
examination of testicular aspirates for T. pallidum. Accordingly, calculation of the CD₅₀ was based on serological results. The CD₅₀ for penicillin was 0.8 μmol or 0.29 mg/kg; that for ceftriaxone was 1.45 μmol or 0.96 mg/kg.

Rabbit deaths occurring during this study were not related to either the drugs or the drug dosages. Three rabbits died from Pasteurella multocida infections, and the other rabbits died during blood collection.

At 4 h after a single dose of 5 μmol of ceftriaxone per kg, T. pallidum rapidly disappeared from orchitic testes. Transmission electron microscopic examination of these treponemes revealed cells into which phosphotungstic acid (PTA) had penetrated, giving them a granular appearance (Fig. 1), whereas spirochetes from untreated rabbits were impervious to PTA and had a smooth appearance (Fig. 2). Examination of ceftriaxone-treated T. pallidum with the scanning electron microscope disclosed blebbing of the outer cell envelope (Fig. 3) which was not seen on untreated cells (Fig. 4).

FIG. 2. Transmission electron micrograph of negatively stained T. pallidum (Nichols strain) from an untreated rabbit. Note the homogeneous appearance of the cell body, indicating no PTA penetration. Bar, 0.5 μm.

DISCUSSION

Our results show that ceftriaxone, a new semisynthetic parenteral cephalosporin, is effective against experimentally induced syphilis in rabbits. The low CD₅₀ of 1.45 μmol or 0.96 mg/kg is probably due in part to the long plasma elimination half-life of the drug of 8 h (9). Although ceftriaxone had a high level of antitreponemal activity, it was somewhat lower than that of penicillin G, which had a CD₅₀ of 0.8 μmol/kg (0.29 mg or 480 U/kg). These results verify the exceptional susceptibility of T. pallidum to penicillin and are similar to the CD₅₀ values of 1.7 to 3.4 μmol/kg (0.6 to 1.2 mg or 1,000 to 2,000 U/kg) for this experimental model reported by Arnold et al. (1).

The presumed mechanism of action of β-lactam antibiotics such as ceftriaxone is inhibition of peptidoglycan synthesis. The penetration of PTA into the outer envelope of ceftriaxone-treated T. pallidum cells probably reflects dam-
age to this cell component. The resulting disruption of cellular function is manifested as blebbing of the cell outer envelope, which is peripheral to and surrounds the peptidoglycan layer. Blebbing of the spirochete outer envelope usually precedes cell death (5).

As dual infections with Neisseria gonorrhoeae and T. pallidum occur, it would be advantageous to be able to treat penicillin-resistant strains of N. gonorrhoeae with a drug effective against both bacteria. Spectinomycin, presently the favored alternative to penicillin for treating gonorrhoea, has poor antitreponemal activity (3). Preliminary studies suggest that a single dose of between 50 and 125 mg of ceftriaxone per kg is adequate for the treatment of uncomplicated gonorrhoea (4). In vitro susceptibility tests have shown that the minimal inhibitory concentration of ceftriaxone for β-lactamase-producing N. gonorrhoeae is approximately 0.01 μg/ml (10). These observations and the results of the current study suggest that ceftriaxone would be effective as a single-dose regimen against mixed infections with T. pallidum and β-lactamase-positive N. gonorrhoeae.

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