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Effects of Cefetamet (Ro 15-8074) on *Treponema pallidum* and Experimental Syphilis

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Cefetamet pivoxil (Ro 15-8075) is a newly developed, expanded-spectrum cephalosporin that is orally active. In vitro, the active form, cefetamet (Ro 15-8074), at a concentration of 0.05 μg/ml killed and lysed *Treponema pallidum*. Rabbit serum did not diminish its effectiveness. The antibiotic rapidly entered the circulation following intramuscular injection into rabbits, attaining its highest levels of 24 to 37 μg/ml within 10 to 30 min. Animals were infected intradermally with *T. pallidum* and then treated with different doses of cefetamet. Accelerated healing was detected following treatment with 15 and 30 mg/kg of body weight. The antibiotic was also effective in killing organisms that had disseminated to distant tissues. In three separate sets of experiments, rabbits were infected with treponemes and then treated with cefetamet intramuscularly at 1, 15, or 30 mg/kg as follows: (i) after lesions had just become clinically apparent, (ii) after lesions were enlarged and well developed, or (iii) prior to the appearance of clinical lesions. Antibiotic effectiveness was determined by sacrificing the animals 1 week after antibiotic treatment and examining splenic tissue for residual, disseminated treponemes. Cefetamet was treponemicidal in all three situations. Maximum effects occurred when the antibiotic was injected before lesions had become clinically apparent (incubation period). These results suggest that cefetamet pivoxil might be useful for treating syphilitic infections.

Cefetamet pivoxil is a newly developed, orally active antibiotic ester with an expanded spectrum and increased antibacterial activity. In vitro, the active metabolite cefetamet is especially effective against gram-negative microorganisms; it is also active against a few gram-positive organisms (1, 4, 5). In vivo, cefetamet has been successfully used to treat pyelonephritis in rats and septicemia in mice (1). In clinical trials with humans (3), cefetamet pivoxil cured bacterial infections that caused acute urethritis, acute cystitis, chronic bronchitis, acute sinusitis, and acute otitis media.

Sexually transmitted diseases continue to be a worldwide problem, especially in undeveloped countries with poor health facilities. Unfortunately, there is an increasing frequency of multiple infections within individual patients; for example, patients with gonorrhea also have syphilis. The incubation period for gonococcal urethritis is 2 to 8 days, compared with 10 to 90 days for primary syphilis. The painful nature of gonococcal symptoms forces a patient to seek treatment prior to the emergence of syphilitic manifestations. Thus, treatment is initiated without awareness of the incubatory, concurrent infection.

Parenteral β-lactams remain the drug of choice for treating both gonorrhea and syphilis. However, improved, preferably orally active, broad-spectrum antibiotics are desirable. Cefetamet kills *Neisseria gonorrhoeae* in vitro, as shown by the evaluation of 410 clinical isolates; a number of these isolates were penicillin resistant (4). The MIC ranged between 0.001 and 0.250 μg/ml. These findings were confirmed clinically. Eighty-six consecutive male patients with acute gonococcal urethritis were successfully treated with one oral dose of cefetamet pivoxil at 1,500 or 1,200 mg (3). The success rate was 100%. Lower doses were almost as fully effective.

Studies need to be directed to the effects of cefetamet on *Treponema pallidum*. The purposes of the research described in this article were to investigate whether cefetamet could kill treponemes in vitro and to determine whether cefetamet could cure experimental syphilis in rabbits. Particular attention was given to establishing the minimal effective concentrations of cefetamet.

MATERIALS AND METHODS

***T. pallidum*.** The Nichols strain was maintained by testicular passage in adult mixed-breed rabbits weighing 2 to 3 kg (6). Each testis was injected with 2×10^7 to 4×10^7 organisms. After developing a palpable orchitis in 9 to 11 days, animals were sacrificed. Testicular tissue was washed in saline, minced with scissors, and extracted in medium containing McCoy's 5A modified medium supplemented with 10% or 60% heat-inactivated (56°C, 30 min) rabbit serum and 1 mM dithiothreitol. After 20 min on a rotary shaker, the suspension was centrifuged at 1,000 × *g* for 10 min to sediment tissue cells and debris. Organisms in the supernatant were adjusted to appropriate concentrations by using a Petroff-Hausser counting chamber.

In vitro experiments. *T. pallidum* was adjusted to 2×10^7 to 2.5×10^7 organisms per ml. Different concentrations of cefetamet were diluted in extraction medium serum and added to the treponemes. After 24 h at 37°C in an environment of 5% carbon dioxide, 2.5% oxygen, and 92.5% nitrogen, the percentage of motile organisms was assessed and both live and dead organisms were counted (11).

In vivo experiments. Two different approaches were used to determine the effects of cefetamet on infection. In approach 1, skin lesions were monitored. Adult female rabbits were injected intradermally with viable *T. pallidum*. Two weeks later, skin lesions were clinically apparent and animals were treated once intramuscularly with different doses of cefetamet. As a positive control, penicillin G at 23,000 U/kg was injected into a separate group of rabbits; this concentration cures rabbit syphilis (6, 13). As a negative control, rabbits were not treated and were observed over the

TABLE 1. In vitro killing and lysis of treponemes by cefetamet

Cefetamet concn (μg/ml)	Results[a] with:			
	10% Rabbit serum		60% Rabbit serum	
	% Motility	Treponemes (10^6/ml)	% Motility	Treponemes (10^6/ml)
0	99 ± 0.6	19.6 ± 2.6	87 ± 7.0	24.3 ± 2.6
0.1	8 ± 2.3	8.6 ± 1.6	22 ± 8.7	14.9 ± 1.9
0.05	53 ± 9.4	12.6 ± 2.4	41 ± 10.8	18.2 ± 2.2
0.01	88 ± 2.0	14.8 ± 1.8	69 ± 6.2	21.4 ± 1.7
0.005	97 ± 0.6	16.1 ± 2.6	81 ± 6.1	22.5 ± 2.0

[a] Results are expressed as the means ± the standard errors of the means for seven separate experiments after 24 h of incubation.

next 3 weeks to monitor either continued lesion development or accelerated healing. In approach 2, dissemination of treponemes was monitored. Rabbits were infected intradermally and after various times were treated with penicillin or cefetamet once intramuscularly. To quantitate spread of *T. pallidum* to distant tissues, rabbits were sacrificed 7 days following antibiotic therapy. Cerebrospinal fluid (CSF) was obtained by removing the base of the skull to expose the spinal cord. Needle aspiration produced 0.5 to 1.0 ml of CSF. Spleens were also removed, washed, cut and minced, and extracted in 10 ml of medium for 20 min by using a rotary shaker. The extracts were centrifuged at 1,000 × *g* for 10 min to sediment splenic cells. Pooled samples of the supernatants or pooled samples of CSF were injected intradermally into two normal rabbits. The development of skin lesions indicated the presence of treponemes within these splenic extracts or CSF samples. The incubation period is equivalent to the time required to develop initial clinical manifestations. It is inversely related to the quantity of organisms infected (8, 13). Representative lesions were aspirated just prior to ulceration; aspirates contained 1 to 5 actively motile spirocytes per 40× field, indicating their syphilitic nature.

Antibiotics. Cefetamet (Ro 15-8074) was supplied by F. Hoffmann-La Roche Ltd. (Basel, Switzerland). Potency was equivalent to 896 μg/mg. Cefetamet was dissolved in physiologic saline. Cefetamet in plasma was kindly analyzed by J. Kneer, Pharmacokinetics Department, F. Hoffmann-La Roche, Ltd., by using a high-pressure liquid chromatography (HPLC) technique specifically developed for this antibiotic (14). The lower limit of sensitivity for this assay is 0.5 μg of cefetamet per ml. Penicillin G (potassium salt) was purchased from Sigma (St. Louis, Mo.) at a concentration of 1,525 U/mg; it was dissolved in physiologic saline.

RESULTS

In vitro effects. Cefetamet was initially tested at concentrations of 10, 1, and 0.1 μg/ml. Treponemes were suspended in medium containing 10% rabbit serum. After incubation for 24 h, motility, as an indicator of viability, and treponemal numbers, as a function of antibiotic-induced lysis, were determined. Killing and lysis were detected at all three concentrations. Cefetamet was then tested at lower levels of 0.1, 0.05, 0.01, and 0.005 μg/ml. The data for seven separate experiments are summarized in Table 1. Treponemes were killed and lysed at concentrations as low as 0.05 μg/ml.

T. pallidum survives better in the presence of higher serum concentrations (10). This, in turn, might partially abrogate the treponemicidal activity of cefetamet. To this end, similar experiments were performed with organisms suspended in 60% rabbit serum. The data from seven separate experiments with this serum are also presented in Table 1. Importantly, the killing and lytic potential of this antibiotic were not decreased by the higher rabbit serum content. In fact, slightly better killing was apparent in the presence of 60% serum.

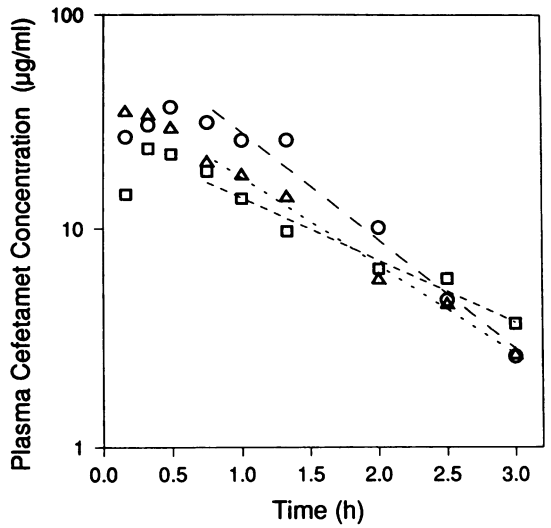

FIG. 1. Cefetamet was injected intramuscularly at 15 mg/kg into three rabbits. Plasma was drawn after 10, 20, 30, 45, 60, 90, 120, 150, and 180 min. High-performance was used to determine cefetamet levels. Data are presented as the corresponding best fit for each animal. Each symbol represents the results for one rabbit.

One additional negative control was tested. To be effective, β-lactams, including cefetamet, require viable organisms that are actively metabolizing. Treponemes were killed by heat inactivation (56°C for 30 min) and then incubated with cefetamet at 0.1, 0.05, 0.01, and 0.005 μg/ml. After 24 h, treponemal numbers were determined as a function of lysis. At each of the four antibiotic concentrations, numbers were identical to those of controls not containing the antibiotic. Thus, nonviable organisms were not lysed by the cefetamet preparations.

Pharmacokinetic studies. Experiments were performed to determine bloodstream entry of cefetamet. Three rabbits were injected intramuscularly with 15 mg/kg. Plasma samples were tested after 10, 20, 30, 45, 60, 90, 120, 150, and 180 min. Pharmacokinetic data are presented as the corresponding best fit for each of the three animals in Fig. 1. There were only slight variations among the rabbits. The highest levels of antibiotic (37, 24, and 36 μg/ml) were achieved within 10 to 30 min. Thereafter, there was a steady, biphasic decrease in antibiotic concentration. After 180 min, cefetamet in plasma averaged between 3 and 4 μg/ml, a level that far exceeds the minimal bactericidal concentration required to kill *T. pallidum* in vitro (0.05 μg/ml).

In vivo infection. Ten animals were infected intradermally with 10^6 treponemes per site at four sites per rabbit. After 14 days, all 40 sites exhibited typical, well-developed, ulcerated lesions 14 to 18 mm in diameter. Animals were separated into five groups. Group 1, the negative control, was not injected with antibiotics. Group 2, the positive control, was injected with penicillin. Groups 3, 4, and 5 were injected with cefetamet at doses of 1, 15, and 30 mg/kg, respectively. Lesions were then observed for either continued development or accelerated healing over the next 2 weeks.

In the negative control group, lesions continued to increase in size and severity. In the positive control group treated with penicillin and in the group treated with the highest dose of cefetamet (30 mg/kg), accelerated healing was apparent within 7 to 10 days. In the group treated with the low dose of cefetamet (1 mg/kg), lesions continued to develop in a fashion identical to that of lesions to within the negative control group. In the group given the intermediate dose of cefetamet (15 mg/kg), some healing was apparent but it was not as pronounced as in the groups injected with penicillin and cefetamet at 30 mg/kg.

Further research involved similar experimental procedures and determination of rapid plasma reaginic antibody (RPR) titers in serum. Levels of reaginic antibody parallel the infectious process in that titers are high when lesions are present and, correspondingly, titers decrease with healing (9). Thus, declining titers suggest antibiotic effectiveness in curing the infections. Five rabbits were infected intradermally. After lesions were well developed, cefetamet was injected once intramuscularly at 0, 1, 5, 15, or 30 mg/kg. RPR titers were determined just prior to and 2 weeks after antibiotic therapy. Titers decreased only at the two highest concentrations, 15 and 30 mg of cefetamet per kg. A second group of five rabbits were infected in a similar fashion and given cefetamet at 0, 0.1, 0.5, 1, or 5 mg/kg. RPR titers did not decrease in any of the animals in this second group. These serologic findings confirmed earlier observations of accelerated healing of dermal lesions with cefetamet at 30 and 15 mg/kg.

Because syphilis is a disseminated infection even in its primary stage, and since dissemination eventually kills the patient, antibiotic effectiveness was characterized in terms of killing disseminated treponemes. Rabbits were infected intradermally with *T. pallidum*. At various times postinfection, animals were injected with the antibiotics; they were sacrificed 1 week later. Pooled samples of CSF or pooled samples of splenic extracts were then injected intradermally in triplicate into two new rabbits.

Three separate protocols were used to reflect antibiotic therapy for patients seeking treatment at various times postinfection. For protocol 1, 25 rabbits were infected intradermally at four sites with 10^4 treponemes per site. Lesions began to appear approximately 12 days later. On day 15, almost all of the sites exhibited very early clinical lesions. At this time, the rabbits were divided into five groups of five animals per group were given injections as follows: group 1, placebo; group 2, penicillin; group 3, cefetamet at 1 mg/kg; group 4, cefetamet at 15 mg/kg; group 5, cefetamet at 30 mg/kg. Rabbits were sacrificed 1 week later, and splenic extracts and CSF were tested for the presence of virulent treponemes.

Table 2 summarizes the results. For the placebo, all six sites injected with splenic extracts developed syphilitic lesions, with an average incubation period of 19.2 days. Penicillin was very effective, and none of the six injected sites developed lesions. For cefetamet at 1 and 15 mg/kg, all 12 sites developed lesions; some treponemicidal activity was apparent, as shown by the 5-day extensions in the incubation periods. This corresponds to at least a 1-log-unit decrease in treponemal numbers within the splenic tissues. For cefetamet at 30 mg/kg, all six sites developed lesions, but better treponemicidal activity was apparent on the basis of longer incubation periods which averaged 13 days beyond that of the placebo. This corresponds to at least a 3-log-unit decrease in treponemal numbers. All CSF samples from the 25 rabbits failed to induce skin lesions, indicating the lack of viable *T. pallidum*.

TABLE 2. Effects of cefetamet on disseminated treponemes when administered at the time of early clinical manifestations

Treatment and dose	Rabbit	Incubation period[a]		No. of sites positive/no. of sites injected
		No. of days	Average	
Placebo	1	18, 18, 20	19.2 ± 0.4	6/6
	2	20, 19, 20		
Penicillin, 23,000 U/kg	1			0/6
	2			
Cefetamet				
1 mg/kg	1	26, 22, 24	24.3 ± 0.5	6/6
	2	24, 24, 26		
15 mg/kg	1	29, 26, 26	24.5 ± 1.2	6/6
	2	22, 22, 22		
30 mg/kg	1	24, 24, 24	32.2 ± 3.7	6/6
	2	42, 42, 37		

[a] Incubation period is the time required to develop the initial clinical manifestations of erythema and induration. Each value is for one pooled site. Averages are expressed as the means ± the standard errors of the means for six sites per group with pools of five splenic extracts per group.

For protocol 2, 25 rabbits were injected intradermally at four sites with 10^6 organisms per ml. Lesions began to emerge at day 4. On day 30, all sites exhibited well-developed, ulcerated lesions with large diameters. At this time, animals were separated into five groups and treated with placebo, penicillin, or cefetamet at 1, 15, or 30 mg/kg. Animals were sacrificed 1 week later, and splenic extracts and CSF samples were injected intradermally into two new rabbits.

Table 3 presents the results. For the placebo group, all six sites developed lesions and the average incubation period was 25 days. Penicillin was very effective, and no lesions were detected at any of the six sites. For cefetamet at 1 mg/kg, no therapeutic effect was apparent; all sites developed lesions with an average incubation period of 22 days. In contrast, the two higher doses of cefetamet were as protective as penicillin in that lesions did not develop at any of the 12 sites injected with the splenic extracts. All CSF samples from the 25 rabbits were negative for *T. pallidum*.

TABLE 3. Effects of cefetamet on disseminated treponemes when administered after lesions had become well developed

Treatment and dose	Rabbit	Incubation period[a]		No. of sites positive/no. of sites injected
		No. of days	Average	
Placebo	1	24, 25, 24	25.5 ± 0.7	6/6
	2	26, 26, 28		
Penicillin, 23,000 U/kg	1			0/6
	2			
Cefetamet				
1 mg/kg	1	22, 22, 22	22.1 ± 0.2	6/6
	2	22, 22, 23		
15 mg/kg	1			0/6
	2			
30 mg/kg	1			0/6
	2			

[a] Incubation period is the time required to develop the initial clinical manifestations of erythema and induration. Each value is for one pooled site. Averages are expressed as the means ± the standard errors of the means for six sites per group with pools of five splenic extracts per group.

TABLE 4. Effects of cefetamet on disseminated treponemes when administered prior to the appearance of clinical manifestations

Treatment and dose	Rabbit	Incubation period[a]		No. of sites positive/no. of sites injected
		No. of days	Average	
Placebo	1	32, 22, 26	24.3 ± 1.7	6/6
	2	22, 22, 22		
Penicillin, 23,000 U/kg	1			0/6
	2			
Cefetamet				
1 mg/kg	1			0/6
	2			
15 mg/kg	1			0/6
	2			
30 mg/kg	1			0/6
	2			

[a] Incubation period is the time required to develop the initial clinical manifestations of erythema and induration. Each value is for one pooled site. Averages are expressed as the means ± the standard errors of the means for six sites per group with pools of five splenic extracts per group.

For protocol 3, 25 rabbits were infected intradermally at four sites with 10^4 treponemes per site. The incubation period for this inoculum should be approximately 12 days. At 9 days postinfection, no lesions had yet appeared. At this time, five groups were treated with placebo, penicillin, or one of the three concentrations of cefetamet. Animals were sacrificed, and splenic extracts and CSF were assessed for residual *T. pallidum*.

Table 4 presents the results for the splenic extracts. The placebo group developed lesions at all six sites, with an incubation period of 24 days. Penicillin was again very effective, and no lesions were observed. Cefetamet at all three concentrations effectively killed the treponemes, and none were detected in these pooled extracts. All CSF samples from the 25 rabbits were again negative and did not induce lesions.

Table 5 summarizes the results for antibiotic effectiveness for each of the three different protocols involving different times of intradermal infection (Tables 2, 3, and 4).

DISCUSSION

Cefetamet is active against a large number of gram-negative microorganisms; at least 22 different species have been shown to be susceptible (1, 4, 5). *T. pallidum* can now be added to this list. Cefetamet concentrations in the range of 0.05 μg/ml killed approximately half of the treponemes within 24 h. Lysis was also apparent, and in accord with other antibiotics that inhibit wall synthesis, cefetamet lysed viable but not dead treponemes, indicating a role for active bacterial metabolism. In addition, high serum concentrations, which are known to enhance treponemal survival, did not adversely influence the treponemicidal activity of cefetamet.

TABLE 5. Summary of the effects of cefetamet on primary syphilitic lesions

Treatment and dose	Status of syphilitic infection[a]		
	Early	Well-developed	Incubating
Penicillin, 23,000 U/kg	+++	+++	+++
Cefetamet			
1 mg/kg	+	0	+++
15 mg/kg	+	+++	+++
30 mg/kg	++	+++	+++

[a] Symbols: +++, very effective killing; ++, intermediate killing; +, minimal killing; 0, lack of killing compared with the placebo control.

Pharmacokinetic studies of cefetamet pivoxil absorption have been performed with adult humans (12). Following oral ingestion of 500 mg, peak levels of 4 μg of cefetamet per ml in serum were attained. In comparable studies, rabbits were injected intramuscularly with 15 mg of cefetamet per kg, a similar dose on a per weight basis. Peak levels of 24 to 37 μg/ml in plasma were attained within 10 to 30 min. After 3 h, levels had decreased to 3 to 4 μg/ml, a concentration still well above that required for in vitro killing. Further experiments with rabbits and with different dosing regimens will be needed to determine the blood concentration time course and the terminal elimination half-life. Such studies will suggest an appropriate dosing schedule for treating human syphilis.

Our studies also demonstrated that cefetamet readily cured rabbits infected with *T. pallidum*. In the preliminary experiments, we attempted to determine the lower level of antibiotic effectiveness. Accordingly, different cefetamet concentrations were injected into rabbits that already had well-developed lesions. The marker for effectiveness was accelerated healing combined with decreasing RPR antibody titers. Cefetamet was treponemicidal at the lower level of 15 mg/kg.

Lesion healing as a marker for antibiotic effectiveness has two disadvantages. First, it can be very subjective. Syphilitic infections spontaneously heal within 3 to 4 weeks. This makes it somewhat difficult to determine accelerated healing, especially when using much lower doses of antibiotic. Second, lesion healing does not always indicate total cure. Syphilis is a localized and a disseminated infection. Immediately after infecting the host, *T. pallidum* enters the lymphatics and the circulation and disseminates to distant tissues. Thus, it is important to further define antibiotic effectiveness in terms of killing disseminated organisms. This key point was emphasized by the recent problems in treating patients with AIDS and concurrent syphilis (2, 7). The standard penicillin regimen was used and the clinical symptoms of syphilis rapidly disappeared, suggesting effective therapy. Within 6 to 12 months, however, these treated patients developed tertiary-stage neurosyphilis. This indicated survival of at least some disseminated treponemes.

On the basis of the above arguments, antibiotic effectiveness is best defined by infecting rabbits, treating them, and then sacrificing them to quantitate residual organisms within distant tissues such as the spleen and spinal cord. Disseminated *T. pallidum* was readily demonstrated in the different splenic extracts. Unfortunately, all CSF samples from the 75 rabbits were negative. Apparently, in experimental rabbit dermal syphilis, *T. pallidum* either fails to gain access to the spinal column in significant amounts, or if it does, is rapidly inactivated.

The data in Tables 2, 3, and 4 involved cefetamet injections at various times following infection with *T. pallidum*. Three different approaches were used to mimic human primary syphilis. In protocol 1, infected rabbits were treated with antibiotics a few days after the initial manifestations of syphilis. This is analogous to a patient seeking immediate treatment as soon as the infection becomes clinically evident. In protocol 2, dermal infections had progressed to enlarged, ulcerated lesions. At this point, antibiotics were

administered. This protocol mimics human infection in which patients are hesitant to seek treatment and the primary lesion has been present for a number of days. In protocol 3, dermal infections had not yet become clinically apparent at the time of antibiotic therapy (incubating syphilis). This is analogous to patients that have infections concurrent with other sexually transmitted diseases, such as gonorrhea. This latter infection has a much shorter incubation period (2 to 8 days) than syphilis (10 to 90 days), and the painful symptoms of urethritis would necessitate physician care well before syphilis became clinically apparent.

Cefetamet was equivalent to penicillin in eradicating disseminated *T. pallidum* if administered either prior to clinical presentations or at the height of lesion severity. Cefetamet lost some of its potency if given at the time of early clinical manifestations. This could reflect different members of treponemes present in rabbit tissues at various times postinfection. It is well established that peak treponemal concentrations occur at the time of early clinical manifestations. Thereafter, host defenses are stimulated and, although lesion severity continues to increase, treponemal numbers rapidly decline. This is confirmed by the data for the splenic extracts for each control preparation listed in Tables 2, 3, and 4. On the basis of differences in the average incubation periods, maximal treponemal numbers occurred in splenic extracts from animals with early clinical manifestations (day 19.2 for erythema and induration). Since 4-day differences in incubation times until erythema and induration are equivalent to 10-fold differences in treponemal numbers, the splenic extracts from the other two protocols probably contained 20- to 50-fold fewer organisms. Thus, cefetamet should be more effective under these latter two conditions.

In summary, cefetamet readily cured experimental syphilis in rabbits. If these findings can be extended to human syphilis, it would represent a significant therapeutic advantage. The oral effectiveness of this antibiotic makes it far more practical than parenteral penicillin, especially in third world countries that have poor health care facilities. In addition, cefetamet pivoxil, with its broad range of antibacterial activities, could be quite effective in treating patients with multiple sexually transmitted diseases such as syphilis and gonorrhea. The efficacy of this newly developed antibiotic may be directly related to its increased affinity for penicillin-binding proteins, its terminal elimination half-life of more than 2 h in humans, and its greater stability in the presence of β-lactamases (1).

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