Therapy of Staphylococcal Infections in Monkeys

VII. Comparison of Cyclacillin and Nafcillin

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Intravenous inoculation of a penicillin-resistant phage type 80/81 staphylococcus caused lethal infection in seven of eight untreated monkeys. Daily intragastric administration of 50 mg/kg given in two equal morning and afternoon doses of cyclacillin and nafcillin was followed by mortalities of four of four and two of four monkeys, respectively. After 100 mg per kg per day, three of four and one of four monkeys receiving cyclacillin and nafcillin, respectively, died. Thus, mortality in controls and cyclacillin-treated monkeys was seven of eight as compared to three of eight after nafcillin treatment. Although the staphylococcus was more resistant to cyclacillin (minimal inhibitory concentration = 7.80 μg/ml) than to nafcillin (minimal inhibitory concentration = 0.31 μg/ml), regular rapid absorption and high levels of the former suggested potential efficacy. However, the similar mortality in cyclacillin-treated and control monkeys indicated that the in vitro data did not, in this instance, conform to the in vivo observations.

Previous studies from this laboratory (1, 7–9, 11, 12) have demonstrated that oral administration of certain antistaphylococcal antibiotics at 50 to 100 mg/kg per kg per day can be curative in potentially lethal infections in monkeys challenged with a penicillin-resistant staphylococcus. This model allows a critical evaluation of comparative in vitro and in vivo effects under controlled conditions simulating human sepsis. Studies in mice (2, 13) infected with a penicillin-resistant staphylococcus showed that cyclacillin, an aminoalicyclic penicillin [6-(1-aminoacyclohexanecarboxamido) penicillanic acid], and nafcillin had “equivalent orders of activity.” The in vivo activity of cyclacillin was greater than anticipated from in vitro data (2, 6, 13). It is the purpose of this report to compare cyclacillin and nafcillin in the therapy of staphylococcal infections in monkeys.

MATERIALS AND METHODS

Twenty-four fully conditioned, young adult monkeys (Macaca mulatta) weighing 3.1 to 4.2 kg were used. Base-line observations included physical examinations and serologic and bacteriologic studies for 2 weeks prior to intravenous (saphenous) challenge with a penicillin-resistant Staphylococcus aureus, phage type 80/81 as described previously (1, 7–12). Tube dilution sensitivity tests showed that minimal inhibitory concentrations (MIC) of cyclacillin and nafcillin for the staphylococcus were 7.80 and 0.31 μg/ml, respectively. Therapy was instituted 16 hr after challenge when monkeys were extremely lethargic, weak, and anorectic. Cyclacillin and nafcillin powders, kindly provided by R. E. Damiano, were dissolved in distilled water immediately before use, and were given intragastrically through a no. 14 disposable plastic urethral catheter attached to a 10-ml syringe. Daily doses of 50 mg/kg (experiment 1) and 100 mg/kg (experiment 2) were divided equally and given at 8:00 AM and 5:00 PM for 12 days; infected control monkeys received only distilled water. The single daily food offering was withheld for at least 1 hr after the 8:00 AM dose. Monkeys were examined at least twice daily for 3 weeks after challenge and daily thereafter for at least 3 months. Laboratory studies included blood cultures, C-reactive protein (CRP) tests, blood urea nitrogen (BUN) levels, serum antibacterial activity (ABA) against the challenge staphylococcus, and serum antibiotic levels in micrograms per milliliter as determined by the conventional plate assay with Sarcina lutea (ATCC no. 9341) as the test organism.

Serum ABA was measured as previously described (3, 4). In brief, serial twofold dilutions of serum were prepared in 0.5-ml amounts of Trypticase soy broth (TSB, BBBL). Each tube was inoculated with 0.05 ml of a 1:1,000 dilution in TSB of a 6-hr-old TSB culture of the challenge staphylococcus. Tests were incubated at 37 C for 16 to 18 hr; inhibitory end points were read as the greatest serum dilution showing no growth on visual examination in a good light.

MIC values of the two antibiotics for the staphylococcus were established by diluting cyclacillin and nafcillin laboratory standards in TSB. The inoculum
and incubation and reading of tests were the same as in serum ABA titrations.

Potency of the cyclacillin and nafcillin powders used in treating monkeys was compared to that of the two laboratory standards. With both antibiotics, zone sizes produced on assay plates by equal amounts of the two preparations were not significantly different. In addition, MIC values of both cyclacillin and nafcillin for the challenge staphylococcus were the same with both preparations.

Results

In experiment 1, 12 monkeys were challenged intravenously with 4.6 × 10⁶ staphylococci. Two groups of four monkeys each were treated with 50 mg per kg per day of cyclacillin and nafcillin, respectively; four monkeys served as untreated controls.

All 12 monkeys were acutely ill when therapy was instituted 16 hr postchallenge; the three groups were similar in this respect. The four control monkeys became progressively worse, and all were dead by the afternoon of day 2. At autopsy, hemorrhage and congestion of the lungs, pericardial effusion, and splenomegaly were the main findings, and phage type 80/81 staphylo cocci were isolated from heart blood and all major organs of all four monkeys.

One of four monkeys treated with cyclacillin did not respond to therapy and died on day 2 after having received only two doses. The remaining three monkeys showed little or no change during day 1, became worse on day 2, and died on days 3, 4, and 7, respectively. Positive blood cultures (phage type 80/81 staphylococci) and CRP tests were observed up to death in all three. Gross pathology in all four cyclacillin-treated monkeys was similar to that in untreated controls with the exception that the monkey that died on day 4 also exhibited multiple myocardial abscesses which yielded staphylococci when cultured. Phage type 80/81 staphylococci were isolated at autopsy from heart blood and all major organs of all four monkeys.

Two of four monkeys given nafcillin died on days 4 and 5, respectively. Clinical, laboratory, and autopsy findings were similar to those in

Table 1. Serum antibacterial activity (ABA), antibiotic levels, and blood urea nitrogen (BUN) levels in monkeys treated with cyclacillin and nafcillin after intravenous challenge with staphylococci

| Expt | Antibiotic | Dose (mg/kg/day) | Monkey | Serum ABA* on day | Antibiotic level (µg/ml)b on day | BUNc on day |
|------|------------|------------------|--------|------------------|-------------------------------|------------|
|      |            |                  |        | 2   | 4   | 9  | 0   | 2 | 4 | 9 | 14 |
| 1    | Cyclacillin| 50               | 222    | 2   | 2   | —  | 18.00| 17.50| —  | 21 | 50 | 48 | —  |
|      |            |                  | 225    | >2  | —   | —  | 0.58 | —   | —  | 22 | 51 | —  | —  |
|      |            |                  | 248    | >2  | —   | —  | 1.00 | —   | —  | 13 | 71 | —  | —  |
| 1    | Nafcillin  | 50               | 219    | <2  | 4   | —  | 0.34 | 1.22 | —  | 13 | 56 | 96 | —  |
|      |            |                  | 273    | 8   | 2   | <2 | 2.00 | 0.73 | <0.01| 25 | 118 | 41 | 14 |
|      |            |                  | 282    | 4   | 32  | —  | 2.00 | 6.50 | —   | 19 | 94 | 174| —  |
|      |            |                  | 283    | <2  | <2  | <2 | <0.01| 0.01| <0.01| 17 | 40 | 24 | 13 | 20 |
| 2    | Cyclacillin| 100              | 270    | 2   | 2   | <2 | 20.00| 9.90| 7.00| 23 | 27 | 22 | 13 | 15 |
|      |            |                  | 285    | 2   | —   | —  | 14.00| —   | —   | 21 | 84 | —  | —  | —  |
| 2    | Nafcillin  | 100              | 228    | 4   | <2  | <2 | 1.60 | 0.33| <0.01| 16 | 39 | 22 | 19 | 18 |
|      |            |                  | 271    | 2   | 2   | <2 | 0.45 | 0.65| <0.01| 23 | 22 | 21 | 11 | 13 |
|      |            |                  | 280    | 2   | <2  | <2 | 0.66 | 0.43| <0.01| 20 | 42 | 20 | 17 | 14 |
| 2    | Controls   | None             | 240    | 4   | 4   | —  | 15   | 189| 52  | 30 |
|      |            |                  | 241    | 4   | —   | —  | 15   | 31 | 63  | 77 |

* Reciprocal of serum dilution inhibitory for S. aureus 80/81 in tube dilution (broth) test; minimal inhibitory concentrations of cyclacillin and nafcillin for the staphylococcus were 7.80 and 0.31 µg/ml, respectively. Samples were obtained 1 hr after morning dose on therapy days 2, 4 and 9. All samples obtained prior to challenge showed no antibiotic activity.

b Plate assay using S. lutea.

c Mg/100 ml of serum. Normal values in humans 5-25 mg/100 ml.

dash (—) indicates monkey dead, no sample.
untreated controls. One (no. 273, Table 1) of the remaining two monkeys was acutely ill during the first 8 days of therapy, began to improve on day 9, and appeared normal on day 15 and thereafter. Blood cultures were positive for the first 7 days, negative on days 9, 11, and 14, positive again on day 17, but negative subsequently. CRP tests were positive continuously for 17 days. No evidence of clinical relapse was observed after therapy was discontinued; the monkey was normal in appearance and activity on day 17 when the positive blood culture was obtained. The fourth nafcillin-treated monkey (no. 283, Table 1) was acutely ill for 7 days and appeared well after day 13. Blood cultures and CRP tests were positive for 11 and 14 days, respectively.

Thus, in summary, in experiment 1, all four control monkeys were dead by day 2, and four monkeys treated with cyclacillin died on days 2, 3, 4 and 7, respectively. Two of four nafcillin-treated monkeys died on days 4 and 5, respectively; the remaining two were acutely ill for 7 and 8 days, respectively, and appeared fully recovered after days 13 and 14, respectively.

In experiment 2, two groups of four monkeys each were challenged with $4.1 \times 10^6$ staphylococci and treated with 100 mg per kg per day of cyclacillin and nafcillin, respectively. Four similarly challenged monkeys received only distilled water. Two of the four control monkeys died on day 2 and exhibited gross pathology similar to that observed in controls in experiment 1. Staphylococci were isolated from heart blood and all major organs of both monkeys. One (no. 240, Table 1) of the remaining two control monkeys became progressively worse during days 1 and 2 and could not rise from the bottom of the cage on days 3 through 5. It improved slightly on day 6, but remained acutely ill for a total of 23 days. Subsequent recovery was slow, and it did not appear normal until day 42. On day 44, however, it became acutely ill and died on day 45. Blood cultures were positive continuously for 28 days, negative on days 35 and 42, but positive on day 44. Extensive pneumonia was observed; multiple abscesses were noted in lungs, liver, heart, kidney, and spleen, and the specific staphylococcus was isolated from all of these sites. The fourth control monkey (no. 241, Table 1) was acutely ill for 15 days and did not appear normal until day 34. Positive blood cultures and CRP tests were noted for 14 and 21 days, respectively.

Two of four monkeys given cyclacillin died on days 1 and 2, respectively, after having received only one and two doses, respectively, and one of the remaining two died on day 4. At autopsy, gross pathology in all three monkeys was similar to that in control monkeys, and, in addition, multiple myocardial abscesses were observed in the monkey that died on day 4. The abscesses yielded staphylococci on culture, as did heart blood and all other major organs of all three monkeys. One cyclacillin-treated monkey (no. 270, Table 1) survived. It was acutely ill for 8 days and appeared well after day 14. Blood cultures remained positive for 21 days, however, and CRP tests were positive for 17 days.

Only one death occurred in the nafcillin-treated group; one monkey died on day 2 after having been given only two doses. The remaining three monkeys began to show improvement after only 4 to 5 days of therapy. Subsequent recovery was rapid and the three monkeys appeared normal after days 7 (no. 228, Table 1), 8 (no. 271), and 10 (no. 280), respectively. However, monkeys 228, 271, and 280 exhibited positive blood cultures for 17, 14, and 11 days, respectively, and positive CRP tests for 17, 11, and 11 days, respectively.

Thus, in experiment 2, three of four control monkeys died, and one was acutely ill for 15 days; it did not recover fully until day 34. Three of four cyclacillin-treated monkeys died, and the fourth was acutely ill for 8 days and did not appear well until day 15. Only one of four monkeys given nafcillin died. The other three began to improve after only 4 to 5 days of therapy, and all were apparently normal after day 10.

As shown in Table 1, serum ABA titers of only 1:2 or <1:2 were observed in five cyclacillin-treated monkeys that survived long enough to study this aspect. Two monkeys treated with 100 mg per kg per day and one given 50 mg per kg per day exhibited levels of 14.0 to 20.0 µg/ml and ABA titers of 1:2 on day 2, whereas levels of only 0.58 and 1.0 µg/ml and ABA titers of <1:2 were noted in the remaining two monkeys given 50 mg per kg per day. The single surviving monkey (no. 270, Table 1) showed 20.0, 9.9, and 7.0 µg/ml on days 2, 4, and 9, respectively; corresponding ABA titers were 1:2, 1:2, and <1:2, respectively. BUN levels increased from 13 to 22 mg/100 ml prior to challenge to 50 to 84 mg/100 ml on day 2 in the four cyclacillin-treated monkeys that died; BUN levels did not change significantly in the survivor (no. 270). BUN levels were also elevated, particularly on days 4 and 9, in the two untreated control monkeys that survived long enough to study this aspect, as shown in Table 1.

Serum ABA titers in monkeys treated with 50 mg per kg per day of nafcillin were highly variable and ranged from <1:2 to 1:32 as com-
pared to <1:2 to 1:4 in those given 100 mg per kg per day. In general, µg/ml values paralleled ABA titers (Table 1). Detectable amounts of antibiotic were observed on days 2 and 4 only; all five monkeys that survived for as long as 9 days showed ABA titers of <1:2 and <0.01 µg/ml. These results with respect to serum levels of nafcillin closely paralleled increases in BUN. For example, BUN levels were higher on days 2 and 4 in monkeys treated with 50 mg per kg per day than in those given 100 mg per kg per day, as were ABA titers (Table 1). In addition, BUN levels were normal on day 9 in the five surviving monkeys (vide supra) that showed no detectable antibiotic on this day. Specifically, monkey no. 282 had the highest ABA titer (1:32), the highest µg/ml level (6.50), and the greatest increase in BUN (174 mg/100 ml) on day 4. It died later the same day.

**Cyclacillin and nafcillin levels in serum of normal monkeys.** Serum ABA titers and antibiotic levels in µg/ml in two groups of six monkeys, each given a single dose of 25 mg/kg, intragastrically, of cyclacillin and nafcillin, respectively, are shown in Table 2. In those given cyclacillin, peak ABA titers were observed 0.5 or 1 hr after the dose. Titers ranged from 1:2 to 1:8 at 0.5 hr and from 1:2 to 1:4 at 1 hr. At 2 hr, sera from two monkeys exhibited ABA titers of 1:2, whereas sera from the remaining four monkeys showed no activity at a dilution of 1:2. Titers of <1:2 were also noted in all six monkeys at 4 and 8 hr. Sera obtained 0.5 and 1 hr after the dose contained from 10.0 to 28.5 µg/ml and from 7.0 to 15.5 µg/ml, respectively. At 2 hr, µg/ml values were below the MIC of the staphylococcus (7.80 µg/ml) used to challenge monkeys in the therapy studies and ranged from 0.5 to 4.0 µg/ml. At 4 hr, sera from four of the six monkeys showed from 0.10 to 0.84 µg/ml, whereas no zones were produced on assay plates by the remaining two sera or by any of the six sera obtained at 8 hr.

None of the 30 serum samples obtained from monkeys given nafcillin showed any antistaphylococcal activity at a dilution of 1:2. Similarly, no zones were produced on assay plates by any of the 30 sera. Thus, no detectable antibiotic was observed in normal monkeys given a single dose of 25 mg/kg of nafcillin.

**DISCUSSION**

Cyclacillin, when administered by gastric tube at a dose of 25 mg/kg to normal monkeys, resulted in remarkably high peak blood levels. As in previous studies (12), nafcillin was not detected in the sera of normal monkeys after oral administration of 25 mg/kg as a single dose. In infected monkeys wherein blood levels were obtained 1 hr after the morning dose of 25 mg/kg of nafcillin, ABA was noted at dilutions varying from 1:2 to 1:32. The discrepancy between findings in normal and infected monkeys could be related in part to altered excretion. For example, on day 2 in monkeys with the highest BUN levels (94 and 118 mg/100 ml, respectively), ABA values of 1:4 and 1:8, respectively, were detected. On day 4, ABA values of 1:2, 1:4, and 1:32 were observed in monkeys with BUN levels of 41, 96, and 174 mg/100 ml, respectively. The fourth monkey which showed no detectable ABA showed the least alteration from base-line BUN. After 100 mg per kg per day nafcillin dosage, ABA of 1:2 to 1:4 was seen in all three surviving monkeys, and, since BUN levels were modestly increased in two, the demonstrable activity more likely represented the increased dose factor. From the in vitro data relative to the greater sensitivity of the staphylococcus to nafcillin (MIC = 0.31 µg/ml) as compared to the MIC of 7.80 µg/ml for cyclacillin, one would expect lesser mortality in the

### Table 2: Cyclacillin levels in serum of normal monkeys

| Time after cyclacillin dose (hr) | Serum antibacterial activitya of monkey no. | Antibacterial level (µg/ml)b of monkey no. | Mean |
|---------------------------------|--------------------------------------------|--------------------------------------------|------|
|                                 | 0 276 277 278 279 280 281                 | 0 276 277 278 279 280 281                 | 0 276 277 278 279 280 281 |
| 0                               | <2 <2 <2 <2 <2 <2 <2                      | <0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05 | <0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05 |
| 0.5                             | 2 4 2 8 4 4 4                           | 10.0 16.5 11.0 28.5 20.0 21.0 17.8       |      |
| 1                               | 2 4 4 2 2 2 2                           | 11.0 15.5 12.0 11.0 11.0 7.0 11.3        |      |
| 2                               | 2 <2 2 <2 <2 <2 <2                      | 4.0 2.0 2.9 2.7 1.8 0.5 2.3             |      |
| 4                               | <2 <2 <2 <2 <2 <2 <2                    | 0.51 <0.05 0.10 0.16 0.84 <0.05 0.27    |      |
| 8                               | <2 <2 <2 <2 <2 <2 <2                    | <0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05 |      |

a Single dose given by gastric tube: 25 mg/kg.
b Reciprocal of serum dilution inhibitory for *Staphylococcus aureus* 80:81 in tube dilution (broth) test; minimal inhibitory concentration of cyclacillin for the staphylococcus: 7.80 µg/ml.

c Plate assay with *Sarcina lutea*.

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nafcillin-treated group. On the other hand, if one considered the rapid, regular absorption of cycla-
cillin, high blood levels, and evidence of ABA against the staphylococcus in normal monkeys as 
compared to the absence of demonstrable levels and ABA in normal monkeys receiving nafcillin, 
one might expect better in vivo results with cyclacillin. The fact that precise correlation between 
in vitro and in vivo data cannot always be made has been the object of previous discussions (5). 
It is conceivable that higher dosages and more frequent administration of the two antibiotics 
studied could have increased survival rates. However, the doses and time of administration 
employed in these studies were the same as used in antecedent studies (1, 7–9, 11, 12) and 
allow a continuing study of comparative efficacy of antibiotics under a standardized method of 
approach.

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