Studies on Therapy of Staphylococcal Infections in Monkeys II. Comparison of Cloxacillin, Dicloxacillin, and Leucomycin (33014)

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Previous studies from this laboratory have described staphylococcal infections in rhesus monkeys (1) and compared the effectiveness following intragastric administration of 2 macrolide antibiotics, erythromycin and triacetyloleandomycin, with a semisynthetic penicillin, cloxacillin (2). It is the purpose of this report to compare the effect of 2 semisynthetic penicillins to a macrolide antibiotic, leucomycin, about which relatively little has been published in relation to experimental infections (3).

Materials and Methods. Fifty-six young adult monkeys (*Macaca mulatta*) weighing 2.7–4.8 kg were used. Intravenous challenge with a penicillin-resistant *Staphylococcus aureus*, phage type 80/81 was conducted after a 2-week period of base-line physical examinations, hematologic and bacteriologic studies. Therapy with cloxacillin (Tegopen, Bristol, oral solution), dicloxacillin (Veracillin, Ayerst) and leucomycin (Kitamycin, Ayerst) was initiated 16-hours postchallenge when clinical and laboratory evidence of well-established infection was present. Contents of dicloxacillin and leucomycin capsules[1] suspended in water and cloxacillin oral solution were given by gastric tube as described previously (2). The daily dose of 50 mg/kg was divided equally and given at 8:00 a.m. (1 hour before the single daily food offering) and at 5:00 p.m. for 12 days; infected controls received only distilled water. All monkeys were examined at least twice daily after challenge; blood counts, cultures and C-reactive protein (CRP) tests were performed as described previously (1). Complete autopsies were done on all fatally-infected animals.

Tube dilution sensitivity tests (4) showed that minimal inhibitory concentrations of cloxacillin, dicloxacillin, and leucomycin for the staphylococcus were 0.4, 0.4, and 1.6 μg/ml, respectively, and bactericidal concentrations were 0.8, 0.8, and 3.1 μg/ml, respectively.

Results. Experiment 1. Three groups of 4 monkeys each were inoculated intravenously with 3.5×10^{10} staphylococci, and treated with cloxacillin, dicloxacillin, and leucomycin, respectively, for 12 days beginning 16 hours postchallenge. Four monkeys served as untreated controls (Table I).

All 16 monkeys had positive blood cultures and CRP, and were extremely lethargic, weak, and anorectic when therapy was instituted. Two of 4 untreated monkeys became progressively worse and died on days 4 and 13, respectively (Table I). Blood cultures and CRP remained positive in both up to death. The monkey which died on day 4 was febrile the first day only, and hypothermic (94.0–99.0°F) for 2 days before death. Total leukocytes were normal, but the differential count showed 91–95% neutrophiles with 48–62% nonsegmented cells as compared to 33–44% and none, respectively, before challenge. The monkey which died on day 13 was febrile (104–106.8°F) the first 11 days and hypothermic (94.0°F) the day before death. Leukocytosis (20,000–21,000) with 85–93% neutrophiles was noted for 2 days, followed by normal counts at 4–9 days, and leukopenia 2 days before death. Hemoglobin and hematocrit values dropped from baseline of 11.3–12.4 gm and 35.0–37.5%, respectively, to 7.8 gm and 21.0%, respectively, on day 11. At autopsy, hemorrhage and congestion of the lungs, pericardial effusion, and splenomegaly were the principal findings in both untreated monkeys, and staphylococci were isolated from most of the major organs.

[1] Kindly provided by Dr. Michael J. Maffei, Ayerst Laboratories.

TABLE I. Effect of Oral Therapy with Cloxacillin, Dicloxacillin, and Leucomycin on Response of Rhesus Monkeys after Intravenous Challenge with Staphylococci.

Antibiotic[a]	Expt. no.[b]	Mortality	Survival time (days)	Duration (days) in survivors, of: Acute illness	Total illness	Mean
Cloxacillin	1	1/4[c]	4, S, S, S[d]	7, 7, 8	9, 10, 27	
	2	3/4	5, 6, 10, S	5	15	16.6
	3	2/3	4, 12, S	7	22	
Total		6/11				
Dicloxacillin	1	0/4	S, S, S, S	2, 2, 3, 5	7, 9, 24, 9	
	2	3/4	2, 3, 27, S	9	21	14.1
	3	1/3	4, S, S	5, 5	13, 16	
Total		4/11				
Leucomycin	1	1/4	10, S, S, S	10, 22, 27	42, 45, 49	
	2	4/4	1, 2, 3, 5	—	—	35.0
	3	1/3	2, S, S	6, 8	20, 19	
Total		6/11				
Controls	1	2/4	4, 13, S, S	11, 24	46, 52	
	2	4/4	1, 1, 1, 1	—	—	42.7
	3	2/3	4, 4, S	13	30	
Total		8/11				

[a] Fifty mg/kg per day (25 mg/kg (b.i.d.) for 12 days beginning 16 hours postchallenge.

[b] Challenge doses in Expts. 1, 2, and 3 were 3.5×10^{10}, 8.3×10^{10}, and 6.5×10^{10} *S. aureus* 80/81 given intravenously.

[c] No. died per total no.

[d] *S* = survived.

The third untreated monkey was extremely lethargic, weak, and anorectic for 11 days, began to improve slowly, but was not normal until day 47. Intermittent fever was noted through the ninth day, and blood cultures (Table II) and CRP were positive for 28 and 49 days, respectively. Leukocytosis was observed for 14 days, and hemoglobin and hematocrit values fell from 12.3–13.2 gm and 39.0–40.0%, respectively, before challenge to 8.7 gm and 26.0%, respectively, by day 17. Initial weight of 4.1 kg dropped to 2.8 kg on day 31 and at 10 weeks had returned to 3.6 kg. The fourth untreated monkey was acutely ill for 24 days after challenge, still very lethargic on day 42 and not normal until day 53. Fever was present the first day only, but blood cultures and CRP were positive for 21 and 17 days, respectively. Leukocytosis and anemia persisted 11 and 17 days, respectively. The monkey weighed 4.1 kg before challenge and 3.2 and 4.3 kg on days 31 and 67, respectively.

One of 4 monkeys treated with cloxacillin died the fourth day (Table I). Slight fever on the first day was followed by hypothermia until death. Positive blood cultures and CRP, leukocytosis, and anemia were observed to death. Gross pathology was similar to that seen in controls, but staphylococci were recovered from heart blood, only. The 3 remaining monkeys receiving cloxacillin began to improve by days 8–9, and survived. One became afebrile by the second day, but showed no other clinical improvement until the eighth day. It then began to recover rapidly and appeared normal after day 10. Leukocytosis (20,000–34,000) was observed for 7 days, and positive blood cultures and CRP for 11 and 21 days, respectively. Another monkey had intermittent fever for 6 days, began to improve on day 8, was active and alert by day 11 and subse-

TABLE II. Incidence of Positive Blood Cultures in Surviving Monkeys Treated with Cloxacillin, Dicloxacillin and Leucomycin after Intravenous Challenge with Staphylococci.

Day post-challenge	Cloxacillin (50 mg/kg per day[a])					Dicloxacillin (50 mg/kg per day)							Leucomycin (50 mg/kg per day)					Controls		
	41	1	C19	12	52	D13	11	7	36	C41	69	29	16	21	31	5	23	C24	V24	49
1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9	+	+	+	+	+	+	+	—	+	+	+	+	—	+	+	+	+	+	+	+
11	+	—	—	+	+	—	—	—	+	+	+	+	—	+	+	+	+	+	+	+
12	Therapy discontinued																			
14	—	+	—	—	—	—	+	+	+	+	+	—	—	—	+	—	—	+	+	+
17	—	—	—	+	+	—	—	—	+	+	—	—	—	—	—	+	+	+	+	+
21	—	—	—	—	+	—	—	+	+	+	—	—	—	—	—	—	+	+	+	—
24	—	—	—	—	+	—	—	—	—	+	—	—	—	—	—	—	+	—	—	—
28	—	—	—	—	—	—	—	—	—	+	—	—	—	—	—	—	—	+	—	—
35	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
42	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
49	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
56	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

[a] Divided into 2 doses given orally at 8:00 a.m. and 5:00 p.m. for 12 days. Therapy started 16 hours postchallenge.

quently. However, blood cultures positive through day 9 and negative on day 11 were positive again on day 14 (2 days after therapy was discontinued), but negative subsequently. Leukocytosis was observed for 11 days. The third monkey that showed improvement beginning day 9 was only mildly lethargic when therapy was discontinued on day 12. On day 13, however, definite relapse was observed, and the animal was extremely lethargic and weak for 3 days, improved rapidly beginning day 16 and appeared normal by day 28. Slight intermittent fever was noted for 8 days, but blood cultures were negative after day 9. The CRP tests were positive through day 17, but negative thereafter; leukocytosis and anemia were observed for 11 and 17 days, respectively. Weight loss in the 3 surviving monkeys treated with cloxacillin was similar to that observed in the 2 surviving controls.

The 4 monkeys treated with dicloxacillin began to show clinical improvement on days 3, 3, 4 and 6, respectively, and appeared normal by days 8, 10, 25 and 10, respectively (Table I). One of 4 was extremely lethargic, weak, and anorectic for only 2 days, began to improve the third day, and appeared normal by the eighth day. Fever, positive blood cultures and CRP, and leukocytosis were observed for only 4, 9, 9, and 11 days, respectively. Thus, this animal was normal in all parameters studied the day before therapy was discontinued, and remained so thereafter. Similarly, the second monkey showing improvement beginning the third day was afebrile and appeared normal by the tenth day, and positive CRP and leukocytosis were observed for 11 days, only. However, blood cultures were positive through day 14. The third monkey began to recover the sixth day, and was in good condition by the tenth day. Intermittent fever was observed for 4 days and leukocytosis and positive CRP and blood cultures for 9, 11, and 21 days, respectively. Thus, this monkey exhibited positive blood cultures for over a week after it appeared normal, and showed no other laboratory evidence of infection. The fourth monkey receiving dicloxacillin showed improvement on day 4, but

subsequent recovery was slow and not complete until day 25. Fever was observed the third day, only. Blood cultures were positive through day 7, negative days 9 and 11, positive day 14, i.e., 2 days after therapy was discontinued, negative at 17 days, positive again at 21 days, but negative thereafter. Similarly, CRP tests were strongly positive (4–6+) through 11 days, weakly positive (1+) days 14 and 17, 4+ and 2+ days 21 and 24, respectively, but negative after day 28. Leukocytosis was present for 24 days. Weight loss in 4 surviving monkeys given dicloxacillin was similar to that observed in 3 surviving monkeys treated with cloxacillin.

One of 4 monkeys treated with leucomycin did not respond, and died on day 10 (Table I). This monkey exhibited fever the first day only, and hypothermia 2 days before death. Positive blood cultures and CRP, leukocytosis, and anemia were observed to death. Gross pathology was similar to that in untreated monkeys except less hemorrhage and congestion of lungs was noted. Staphylococci were isolated from most of the major organs. One of the remaining 3 given leucomycin showed no improvement the first 3 days, became worse day 4 and was still acutely ill with fever, positive blood cultures and CRP, leukocytosis, and anemia when therapy was discontinued day 12. Its condition fluctuated considerably the next 10 days, and slow recovery beginning day 23 was not complete until day 46. Blood cultures and CRP became negative on days 14 and 21, respectively; leukocytosis and anemia persisted for 24 and 49 days, respectively. Similarly, another monkey showed no improvement the first 2 days of therapy, became worse the third day, and was still acutely ill, although afebrile when therapy was stopped after 12 days. Positive blood cultures and CRP, leukocytosis, and anemia were observed throughout the therapy period. Posttherapy the monkey remained very lethargic and weak until slight improvement beginning day 28 was followed by slow recovery which was complete by day 50. Blood cultures and CRP were positive through day 14; leukocytosis and anemia were observed for 21 and 24 days, respectively. The fourth monkey given leucomycin was afebrile but extremely lethargic, weak, and anorectic the first 10 days of therapy, but showed some improvement by day 11. Subsequent recovery was slow and it did not appear normal until day 43. Positive CRP, leukocytosis, and anemia were observed for 17, 24, and 28 days, respectively, but blood cultures were negative after day 7. Weight loss in 3 surviving monkeys given leucomycin was similar to that in 2 untreated survivors.

Experiment 2. Therapy with 50 mg/kg per day of cloxacillin, dicloxacillin, and leucomycin was instituted in 3 groups of 4 monkeys each, respectively, 16 hours after intravenous challenge with 8.3×10^{10} staphylococci. Four similarly challenged monkeys served as untreated controls.

This dose caused death of all 4 untreated monkeys by the first day after challenge (Table I). Four monkeys treated with leucomycin died on the first (after 2 doses), second, third, and fifth days, respectively. Similarly, 3 of 4 and 2 of 4 given cloxacillin and dicloxacillin, respectively, died on the fifth, sixth, and tenth, and second and third days, respectively. Clinical, laboratory, and autopsy findings in these 13 monkeys were similar to those seen in Expt. 1.

Thus, when therapy was discontinued after 12 days, 3 monkeys were alive. One of 2 given dicloxacillin was acutely ill for 6 days, began to improve day 7, but remained slightly lethargic through day 23. It suddenly became worse on day 24, and died day 27. Intermittent fever, positive blood cultures and CRP, leukocytosis, and anemia were present to death. Gross pathology was similar to that seen in other monkeys and, in addition, the left kidney was twice normal size with numerous hemorrhagic areas in the cortex extending into the subcapsular area and into the pyramids; the right kidney was similarly involved but to a lesser extent. Staphylococci were isolated from both kidneys and most of the major organs. The other monkey treated with dicloxacillin became afebrile the second day, but showed little change the first 9 days of therapy,

began to improve slowly on day 10, but was still very lethargic, and had positive blood cultures and CRP, leukocytosis, and anemia when therapy was discontinued day 12. It continued to improve after day 12 and appeared normal by day 22 although blood cultures and CRP were positive for 28 and 35 days, respectively, and leukocytosis and anemia were observed for 28 and 35 days, respectively.

The surviving monkey given cloxacillin began to improve slowly by day 6 of therapy, was only mildly lethargic and afebrile when treatment was stopped day 12, and appeared normal by day 16. However, positive blood cultures and CRP, leukocytosis, and anemia were observed for 17, 21, 14, and 28 days, respectively.

Experiment 3. Three groups of 3 monkeys each received 6.5×10^{10} staphylococci, intravenously, and were treated with 50 mg/kg per day of cloxacillin, dicloxacillin, and leucomycin, respectively, for 12 days beginning 16 hours after challenge. Three control monkeys were similarly challenged, but not treated.

All 12 monkeys were acutely ill when therapy was instituted; blood cultures and CRP were positive in all. Two of 3 untreated monkeys became progressively worse and died the fourth postchallenge day; the other also became progressively worse, and was prostrate and semicomatose the second day. The third day it was able to rise, but remained extremely weak, lethargic, and anorectic for 13 days, then began to recover slowly and appeared normal by day 31. It was afebrile throughout, but blood cultures and CRP were positive for 17 and 24 days, respectively; leukocytosis and anemia were noted for 14 and 35 days, respectively.

Two (nos. 50, 37) of 3 monkeys receiving cloxacillin died on days 4 and 12, respectively. The third (no. 52), which was febrile for 4 days and acutely ill during the first 7 days of therapy, was very lethargic and still had positive blood cultures and CRP, leukocytosis, and anemia when treatment was discontinued day 12. Subsequent recovery was slow and not complete until day 23. Positive blood cultures and CRP were noted for 24 and 21 days, respectively; leukocyte counts and hematocrits were normal after days 21 and 35, respectively.

One (no. 99) of 3 monkeys given dicloxacillin showed no improvement, and died on the fourth day. One (no. 69) of the remaining 2 was febrile the first 4 days, began to improve day 6, and appeared normal by day 14. Blood cultures and CRP were positive for 14 and 17 days, respectively; leukocytosis and anemia persisted for 14 and 24 days, respectively. The third monkey (no. 29) given dicloxacillin also began to improve the sixth day of therapy, but on the ninth day, an abscess (0.5×2.0 cm) was noted at the site of intravenous (saphenous) inoculation. The lesion opened and drained on days 11 and 12 and staphylococci were isolated. Subsequent healing and recovery were rapid and complete by day 17. Blood cultures and CRP were positive for 11 and 17 days, respectively; leukocytosis was noted from 7–14 days and anemia from 4–17 days.

One of 3 monkeys receiving leucomycin died the second day. The other 2 (nos. 5, 23) remained extremely lethargic, weak, and anorectic for 6 and 8 days, respectively, improved slowly, and appeared well by days 21 and 20, respectively. Monkey 5 was afebrile, but blood cultures and CRP were positive for 17 days, and leukocytosis and anemia were observed for 14 and 24 days, respectively. Monkey 23 showed intermittent fever the first 7 days, but blood cultures and CRP were positive for 24 and 17 days, respectively. Leukocytosis was noted the first 4 days only, but anemia persisted for 35 days.

Although the prime criterion for comparative efficacy was the clinical response to therapy, limited studies were included to determine serum inhibitory levels in Expt. 3. In order to avoid too frequent accessory bleedings during the course of infection, studies were performed from blood obtained the morning of the fourth day just prior to the next dose. This would determine the *lowest* level of antibiotic activity. In only 3 of 7 monkeys was any inhibitory effect noted. The serum of 1 (no. 37) of 2 receiv-

ing cloxacillin inhibited the staphylococcus at a 1:2 dilution. This monkey died on day 12 while the other (no. 52) survived. Undiluted sera of 1 (no. 5) of 2 receiving leucomycin inhibited the staphylococcus; both survived. Undiluted sera of 1 (no. 29) of 3 receiving dicloxacillin inhibited the staphylococcus; it survived as did 1 of the other 2.

Antibiotic levels in normal monkeys. An additional study was conducted in 12 normal monkeys 1 and 2 hours after intragastric administration of 25 mg/kg of antibiotic. Serum from monkeys receiving cloxacillin inhibited the staphylococcus at dilutions of 1:2, 1:2, 1:4, and 1:4, respectively, at 1 and 2 hours. Identical results were obtained following leucomycin. After dicloxacillin, inhibition was observed at 1:4, 1:4, 1:8, and 1:8, respectively, after 1 hour and at 1:4, 1:4, 1:4, and 1:8, respectively, after 2 hours.

Discussion. It is obvious that these experiments were designed to produce a severe, life-threatening infection and therapy was delayed until the monkeys were critically ill. In similar clinical situations one would be hesitant to use oral therapy, and if one did, certainly the drug would be given more frequently than twice daily. Thus, under the conditions of this study, the test antibiotics were assessed in the presence of overwhelming infection. The staphylococcus employed was inhibited *in vitro* by all 3 agents, and cloxacillin and dicloxacillin were similar in this respect, and lesser minimal inhibitory concentrations were necessary than with leucomycin. To compare absorption, studies in normal monkeys showed that serum obtained at 1 and 2 hours after intragastric administration of cloxacillin and leucomycin showed inhibition of the staphylococcus at 1:2 to 1:4 dilutions, and at 1:4 to 1:8 dilutions after dicloxacillin. Serum obtained from survivors on the fourth day prior to the next dose were comparable in absence of or presence of inhibitory properties in the 3 treatment groups. In reference to mortality, 6 of 11 in both the leucomycin- and cloxacillin-treated groups died as compared to 4 of 11 receiving dicloxacillin. Comparable results were also noted in studies of blood cultures. In surviving monkeys, 2 of 5, 2 of 7, and 2 of 5 treated with cloxacillin, dicloxacillin, and leucomycin, respectively, had negative cultures after therapy was discontinued after day 12, and all monkeys in each group had negative cultures after days 24–28 postchallenge. Only in reference to duration of obvious illness were there any distinct differences observed in that the leucomycin-treated animals did not begin to show clinical evidence of recovery as rapidly as did those treated with the semisynthetic penicillins. Mean duration of obvious illness was 16.6, 14.1, and 35.0 days, respectively, in monkeys treated with cloxacillin, dicloxacillin, and leucomycin, respectively. That the latter factor could not be attributed to intolerance to the drug would be supported by preliminary studies in which monkeys were shown to tolerate 14 days of 50 mg/kg of leucomycin without any obvious illness or discomfort.

Conclusions. Daily intragastric administration of 50 mg/kg of cloxacillin, dicloxacillin, and leucomycin beginning 16 hours postintravenous challenge with a penicillin-resistant, phage type 80/81 staphylococcus was followed by comparable results in most parameters measured. Only in duration of obvious clinical illness were differences noted in that leucomycin-treated monkeys did not appear well until 19–45 (mean 35) days after institution of therapy as compared to 9–27 (mean 16.6) and 7–24 (mean 14.1) days, respectively, in those treated with cloxacillin and dicloxacillin, respectively.

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