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Comparison of Clindamycin, Erythromycin, and Methicillin in Streptococcal Infections in Monkeys

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Intravenous inoculation of a group A hemolytic streptococcus caused lethal infections in all of eight untreated monkeys. Intramuscular injections of clindamycin-2-phosphate in a daily dose of 25 mg/kg given in equal morning and afternoon doses for 10 days resulted in survival of all of eight monkeys. Similar results were observed with the same dose schedule of clindamycin hydrochloride given intragastrically; no fatalities occurred among eight monkeys. In monkeys receiving erythromycin stearate intragastrically or methicillin intramuscularly, three of eight and four of eight monkeys, respectively, died. Duration of both illness and positive blood cultures was greater in the erythromycin- and methicillin-treated survivors than in the clindamycin-treated monkeys. The differences in results between clindamycin and erythromycin could not be correlated with serum antibacterial activity levels, which were similar, or with minimal inhibitory concentrations, which were 0.02 μg/ml with both antibiotics. With methicillin, however, the minimal inhibitory concentration was 0.16 μg/ml and serum antibacterial activity varied from titers of less than 1:2 to 1:8. As in previous studies of staphylococcal infections in monkeys with the same antibiotics, in vitro susceptibility data and serum antibacterial activity did not completely correlate with in vivo results.

Previous studies demonstrated similar efficacy with reference to mortality of monkeys with severe staphylococcal sepsis after therapy with intragastric clindamycin hydrochloride or erythromycin stearate or intramuscular clindamycin-2-phosphate or methicillin (1). The purpose of this study was to compare the oral and injectable forms of clindamycin in streptococcal sepsis. As in the staphylococcal study, erythromycin stearate and methicillin were also included for comparative purposes.

MATERIALS AND METHODS

Forty fully conditioned monkeys (*Macaca mulatta*) weighing 2.8 to 3.9 kg were used. Intravenous challenge with the Stollerman T14 strain of *Streptococcus hemolyticus* group A was conducted after a 2-week period of base-line physical examinations and bacteriological and serological studies, as previously described (6, 9, 10). Therapy was initiated 8 hr after challenge when monkeys were lethargic, weak, and anorectic but not acutely ill. The daily dose of 25 mg/kg was divided equally and given by gastric tube (clindamycin hydrochloride and erythromycin stearate) or intramuscularly (clindamycin-2-phosphate and methicillin) at 8:00 AM and 5:00 PM for 10 days; infected controls received distilled water intragastrically or saline intramuscularly. Food was withheld for at least 1 hr after the morning dose. Clindamycin hydrochloride capsules and injectable solution of clindamycin-2-phosphate were kindly provided by R. M. DeHaan; erythromycin stearate tablets and methicillin were purchased from a local pharmacy. Oral suspensions were prepared from tablets and contents of capsules as described previously (1).

Monkeys were examined at least twice daily for 3 weeks after challenge, and then daily thereafter for at least 3 months. Duration of "acute illness" included days in which the monkey was markedly anorectic, lethargic, and weak, and did not respond to stimuli; it would lie in the cage huddled up. "Total illness" included both acute and convalescent periods until the animal again became freely active, vigorous, and alert. Laboratory studies included blood cultures, C-reactive protein (CRP) tests, antistreptolysin O (ASO) titers, blood urea nitrogen (BUN) and serum glutamic-pyruvic transaminase (SGPT) levels, and serum antibacterial activity (ABA) against the challenge streptococcus. Autopsies were performed on all fatally infected animals.

Serum ABA was measured as previously described (2, 3). Minimal inhibitory concentrations (MIC) of clindamycin base, erythromycin base, and methicillin

TABLE 1. *Effect of therapy with clindamycin hydrochloride, clindamycin-2-phosphate, erythromycin stearate, and methicillin on response of rhesus monkeys after intravenous challenge with streptococci*

Antibiotic[a]	Expt	Mortality	Day of death	Acute illness (day)	Total illness (day)
Clindamycin hydrochloride	1	0/4[b]	—	2, 3, 3, 3	3, 4, 4, 4
	2	0/4	—	2, 2, 2, 3	4, 4, 4, 4
	Mean			2.5	3.9
Clindamycin-2-phosphate	1	0/4	—	2, 2, 2, 3	3, 3, 3, 4
	2	0/4	—	2, 3, 3, 5	3, 4, 6, 6
	Mean			2.8	4.0
Erythromycin stearate	1	1/4	1	2, 4, 6	4, 6, 8
	2	2/4	1, 8	4, 4	5, 5
	Mean			4.0	5.6
Methicillin	1	2/4	2, 2	2, 5	3, 6
	2	2/4	1, 1	2, 10	3, 14
	Mean			4.8	6.5
Controls	1	4/4	1, 1, 1, 2	—	—
	2	4/4	1, 1, 1, 1	—	—

[a] A daily dose of 25 mg/kg divided equally and given by gastric tube (clindamycin hydrochloride and erythromycin stearate) or intramuscularly (clindamycin-2-phosphate and methicillin) at 8:00 AM and 5:00 PM for 10 days beginning 8 hr after challenge with the Stollerman T14 strain of *S. hemolyticus* group A. Challenge doses in experiments 1 and 2 were 5.8×10^9 and 4.7×10^9 streptococci, respectively.

[b] Number that died/total number.

for the challenge *Streptococcus* strain used in determining ABA titers were 0.02, 0.02, and 0.16 μg/ml, respectively.

RESULTS

In experiments 1 and 2 (Table 1), the four antibiotics were compared at doses of 25 mg per kg per day in monkeys challenged with 5.8×10^9 and 4.7×10^9 streptococci, respectively. All monkeys in both studies were weak, lethargic, and anorectic when therapy was started 8 hr after challenge. The eight untreated control monkeys became progressively worse, and all were dead by the afternoon of day 2. Hemorrhage and congestion of the lungs, pericardial effusion, and splenomegaly were the main findings at autopsy; group A streptococci were isolated from heart blood and all major organs of all eight monkeys.

All 16 monkeys treated with either of the two clindamycin preparations survived, whereas 3 of 8 and 4 of 8 monkeys given erythromycin stearate and methicillin, respectively, did not respond to therapy and died (Table 1). The eight survivors treated with clindamycin hydrochloride were acutely ill for 2 to 3 days (mean, 2.5 days), recovered rapidly, and appeared normal after 3 to 4 days (mean, 3.9 days). Similarly, acute illness was observed for 2 to 5 days (mean, 2.8 days) in eight monkeys given clindamycin-2-phosphate, and all were apparently fully recovered after 3 to 6 days (mean, 4.0 days).

Two monkeys treated with erythromycin stearate died about 36 hr after challenge after having received two doses of antibiotic. Gross pathology was similar to that observed in untreated control monkeys, and all major organs and heart blood yielded group A streptococci. A third monkey in experiment 2 (no. 31, Table 3) showed no response to therapy during the first 5 days. On day 6, cellulitis involving the upper half of the face and particularly the orbits was observed. In addition, evidence of epistaxis was noted. Group A streptococci were isolated by nasal swab and by needle aspiration of the area of cellulitis. The monkey became worse on day 7 and was moribund at 8:00 AM on day 8 when the morning dose of erythromycin was given; it died about 5 hr later. At autopsy, the lungs were markedly hemorrhagic and the trachea was filled with frothy blood-tinged fluid. None of the major organs or heart blood yielded streptococci when cultured, and blood cultures obtained on days 3, 4, 6, and 8 were negative. As can be seen in Table 3, the high ABA levels may have prevented recovery of the organism. The BUN on day 2 was 134 mg/100 ml and increased to 310 mg/100 ml

on day 6, as compared to 13 mg/100 ml prior to challenge. Serum samples obtained from this monkey on days 6 and 8 *before* the morning dose, i.e., about 16 hr after the afternoon dose on the previous day, showed an ABA titer of 1:16 on both days; no ABA was detected at the same time interval on either day in the other two erythromycin-treated survivors. Thus, three treatment failures were observed in the group of eight monkeys given erythromycin stearate. The five surviving monkeys were extremely weak, lethargic, and anorectic for 2 to 6 days (mean, 4.0 days) and did not recover fully until after 4 to 8 days (mean, 5.6 days).

Four of eight monkeys treated with methicillin did not respond to therapy, and were dead by the afternoon of day 2 after having received from two to four doses (Table 1). Group A streptococci were isolated from all major organs and heart blood of all four monkeys. Two (no. 15 and 36, Tables 2 and 3) of four surviving monkeys began to improve after only 2 days of therapy, and both appeared normal on day 4 and thereafter. The other two monkeys (no. 16 and 35) remained acutely ill for 5 and 10 days, respectively, and did not recover fully until days 7 and 15, respectively.

Positive blood cultures were obtained during the therapy period from only two of eight monkeys treated with clindamycin hydrochloride and from only one of eight monkeys given clindamycin-2-phosphate, as shown in Table 2. Blood cultures were negative in all 16 monkeys after the 10-day treatment period. In contrast, all four surviving methicillin-treated monkeys and three of five monkeys surviving after erythromycin stearate exhibited positive blood cultures while on

TABLE 2. *Incidence of positive blood cultures in surviving monkeys treated with clindamycin hydrochloride, clindamycin-2-phosphate, erythromycin stearate, and methicillin after intravenous challenge with streptococci*

Antibiotic[a]	Expt[b]	Monkey	Blood culture at postchallenge day[c]												
			3	4	6	8	10[d]	14	17	21	24	28	35	42	49, 56
Clindamycin hydrochloride	1 (4)	1	−	−	−	−	−	−	−	−	−	−	−	−	−
		2	−	−	−	−	−	−	−	−	−	−	−	−	−
		3	−	−	−	−	+	−	−	−	−	−	−	−	−
		4	−	−	−	−	−	−	−	−	−	−	−	−	−
	2 (4)	21	−	−	−	−	−	−	−	−	−	−	−	−	−
		22	+	+	−	−	−	−	−	−	−	−	−	−	−
		23	−	−	−	−	−	−	−	−	−	−	−	−	−
		24	−	−	−	−	−	−	−	−	−	−	−	−	−
Clindamycin-2-phosphate	1 (4)	5	−	−	−	−	−	−	−	−	−	−	−	−	−
		6	−	−	−	−	−	−	−	−	−	−	−	−	−
		7	−	−	−	−	−	−	−	−	−	−	−	−	−
		8	−	−	−	−	−	−	−	−	−	−	−	−	−
	2 (4)	25	−	+	−	−	−	−	−	−	−	−	−	−	−
		26	−	−	−	−	−	−	−	−	−	−	−	−	−
		27	−	−	−	−	−	−	−	−	−	−	−	−	−
		28	−	−	−	−	−	−	−	−	−	−	−	−	−
Erythromycin stearate	1 (3)	9	−	−	+	+	+	+	+	−	−	+	−	−	−
		11	−	−	−	−	−	−	−	−	−	−	−	−	−
		12	+	−	−	−	+	−	−	−	−	−	−	−	−
	2 (2)	30	−	−	+	+	+	−	−	+	+	+	+	+	−
		32	−	−	−	−	−	−	−	−	−	−	−	−	−
Methicillin	1 (2)	15	−	+	−	−	−	−	−	−	−	−	−	−	−
		16	+	+	−	−	−	+	−	−	−	−	−	−	−
	2 (2)	35	+	+	+	+	+	+	+	+	+	+	+	+	+[e]
		36	+	+	−	−	−	−	−	−	−	−	−	−	−

[a] Dose of 25 mg per kg per day.
[b] Number in parentheses represents number of survivors.
[c] All blood cultures negative before challenge.
[d] Therapy discontinued on the 10th day.
[e] Blood cultures negative on days 63 and 70.

therapy, and two monkeys in each group also showed positive cultures after day 10. One of the two erythromycin-treated monkeys (no. 9, Table 2) appeared normal on therapy day 5 and thereafter. However, blood cultures were positive continuously from days 6 through 17, negative on days 21 and 24, positive again on day 28, but negative subsequently. Similarly, the other erythromycin-treated monkey (no. 30) appeared well by day 6, but blood cultures were positive on days 6 to 10 and positive again from days 21 to 42, but negative thereafter. Neither of the two monkeys exhibited any evidence of clinical relapse after therapy was discontinued. One of the two methicillin-treated monkeys (no. 16) had a single positive blood culture on day 14 after cultures had been positive on days 3 and 4 and negative on days 6 to 10. The monkey appeared normal after 6 days of therapy, and remained so thereafter. The other methicillin-treated monkey (no. 35) responded poorly to therapy, and did not recover fully until day 15. Blood cultures were positive continuously from day 3 through day 56. Thus, four surviving monkeys given erythromycin stearate or methicillin exhibited positive blood cultures for about 1, 3, 5, and 6 weeks, respectively, after they had apparently recovered fully from the streptococcal infection.

CRP tests were negative after 3 to 8 days of therapy in all 16 monkeys treated with clindamycin hydrochloride or clindamycin-2-phosphate. Positive tests were observed for 3, 8, 9, 10, and 10 days in five survivors given erythromycin stearate and for 3, 3, 8, and 17 days in four survivors given methicillin. Persistence of positive CRP tests generally paralleled duration of illness.

Base-line SGPT levels in the 40 monkeys in the two experiments ranged from 9 to 42 units (mean, 20 units) as compared to 15 to 45 units in humans. Significant changes in SGPT were observed after challenge in only two monkeys. One monkey (no. 31, Tables 2 and 3) treated with erythromycin stearate exhibited levels of 17 units prior to challenge and 177 units on day 6; it died on day 8. As discussed previously, this monkey also showed facial cellulitis, elevated BUN, high serum ABA titers, negative blood cultures, and negative cultures at autopsy. SGPT levels in one of the methicillin-treated monkeys (no. 35) increased from 16 units before challenge to 69, 38, and 28 units on days 6, 14, and 28, respectively. This monkey responded very poorly to therapy and did not appear normal until day 15 (Table 1). Blood cultures were positive continuously through day 56 (Table 2).

No evidence of local reaction at the site of intramuscular inoculation was observed during therapy or thereafter in monkeys treated with clindamycin-2-phosphate or methicillin. One monkey given clindamycin hydrochloride, intragastrically, exhibited mild diarrhea on therapy days 6 and 7 only. The other monkeys treated with oral preparations of clindamycin or erythromycin remained normal in this respect throughout the observation period.

Serum ABA of samples obtained 2 hr after the 8:00 AM dose on therapy days 2 and 9 are shown in Table 3. MIC values of clindamycin base, erythromycin base, and methicillin for the challenge streptococcus were 0.02, 0.02 and 0.16 μg/ml, respectively. Monkeys given clindamycin hydrochloride intragastrically showed ABA titers of 1:128 to 1:1,024 on day 2 and 1:32 to 1:256 on day 9. Comparable high ABA titers were observed at the same time intervals after clindamycin-2-phosphate and erythromycin stearate. In contrast, monkeys receiving methicillin showed less serum ABA; titers of $<$1:2 to 1:8 were observed, as shown in Table 3.

As in previous studies (6, 9, 10) BUN levels were frequently increased as a result of infection on day 2 during the acute illness and returned to normal by day 9 after recovery. Thus, some of the differences in ABA titers in individual monkeys on days 2 and 9 could be attributed, in part, to retention of antibiotics, as shown in Table 3.

Significant increases in ASO titer were noted in all of the five surviving monkeys treated with erythromycin stearate and in three of the four survivors given methicillin. In contrast, only five of eight and four of eight monkeys treated with clindamycin hydrochloride and clindamycin-2-phosphate, respectively, showed significant ASO titer changes. These differences may be related, in part, to the apparently earlier elimination of the streptococcus in the clindamycin-treated monkeys.

DISCUSSION

In a study at this institution to evaluate the efficacy of clindamycin in patients, this antibiotic was found to be especially effective in soft tissue infections (R. J. Fass and S. Saslaw, *in press*). These infections were most often due to hemolytic streptococci, to penicillin-susceptible or resistant staphylococci, or to both streptococci and staphylococci. A previous study in monkeys compared the effect of clindamycin, erythromycin, and methicillin in infections caused by a penicillin-resistant staphylococcus (1). Since staphylococcal and streptococcal infections may at times be confused clinically or coexist, the present studies on streptococcal infection were instituted to compare the same antibiotics employed in the staphylococcal study.

After administration of clindamycin hydro-

TABLE 3. *Serum antibacterial activity and blood urea nitrogen levels in monkeys treated with clindamycin hydrochloride, clindamycin-2-phosphate, erythromycin stearate, and methicillin after intravenous challenge with streptococci*

Expt	Antibiotic[a]	Monkey no.	Day 0 BUN[c]	Day 2[b]		Day 9	
				ABA[d]	BUN	ABA	BUN
1	Clindamycin hydrochloride	1	25	512	27	128	11
		2	20	512	29	256	13
		3	18	1,024	32	256	12
		4	23	1,024	35	128	15
	Clindamycin-2-phosphate	5	23	512	24	256	11
		6	28	1,024	42	256	8
		7	24	1,024	37	256	15
		8	28	1,024	22	256	13
	Erythromycin stearate	9	27	256	32	128	14
		10	21	D1[e]			
		11	22	1,024	122	256	15
		12	19	512	74	256	16
	Methicillin	13	21	D2			
		14	26	D2			
		15	28	<2	42	<2	11
		16	24	8	42	<2	14
2	Clindamycin hydrochloride	21	21	512	42	32	24
		22	23	256	40	128	14
		23	22	256	28	64	20
		24	18	128	22	32	18
	Clindamycin-2-phosphate	25	17	512	38	128	16
		26	20	512	60	256	18
		27	22	512	70	128	20
		28	22	256	20	256	14
	Erythromycin stearate	29	16	D1			
		30	17	128	34	32	16
		31	13	1,024	134	D8	
		32	16	256	36	32	20
	Methicillin	33	23	D1			
		34	23	D1			
		35	16	4	42	2	14
		36	22	2	30	<2	16

[a] Dose of 25 mg per kg per day.
[b] Sample taken 2 hr after morning dose on therapy days 2 and 9.
[c] Blood urea nitrogen (mg/100 ml of serum). Normal values in humans, 5 to 25 mg/100 ml.
[d] Antibacterial activity expressed as reciprocal of serum dilution inhibitory for the challenge streptococcus in broth-dilution test. Minimal inhibitory concentrations of clindamycin base, erythromycin base, and methicillin for the streptococcus were 0.02, 0.02 and 0.16 μg/ml, respectively.
[e] Died on day 1.

chloride intragastrically or clindamycin-2-phosphate intramuscularly in a daily dose of 25 mg/kg, all of eight monkeys in each therapy group survived. At the same dose level, three of eight monkeys receiving intragastric erythromycin and four of eight receiving intramuscular methicillin died. In vitro susceptibility data and serum ABA suggest that comparable results might be expected in the clindamycin- and erythromycin-treated animals, but higher mortality and longer duration of both illness and positive blood cultures were observed in the erythromycin-treated monkeys. In the methicillin-treated group, the higher mortality could be related to the lesser susceptibility of the infecting organism and lower ABA observed in the in vitro studies. However, in the previous study (1) with a penicillin-resistant strain of *Staphylococcus*, the data suggested that methicillin would be the least effective therapeutic agent of the four preparations studied; this prediction was not fulfilled. For example, ABA levels against the staphylococcus were only 1:2 or less at 2 hr compared to the considerably higher ABA observed at this time interval with

clindamycin and erythromycin. Yet, clinical outcome was the same.

Thus, as demonstrated in previous studies on experimental staphylococcal (1, 5, 7, 8, 11, 12) and streptococcal (6, 9, 10) sepsis in monkeys, the data support the general impression that organisms susceptible in vitro will usually be controlled in vivo by appropriately selected antibiotics. However, more evidence is herein added to expand antecedent discussions (4) concerning the significance of serum levels of antibiotics and relative therapeutic effects. Studies of comparative efficacy of antibiotics under controlled conditions in humans with severe life-threatening sepsis are difficult to attain. These experiments were designed to produce a severe lethal infection, and therapy was delayed until the monkeys were critically ill. In similar clinical situations, one would be hesitant to administer antibiotics only twice daily, and particularly by the oral route. However, to avoid variables, the twice daily regimen employed in previous studies has been continued here. If animals survive on this regimen, one can certainly feel confident of an antibiotic's potential efficacy if given more often. If mortalities occur, one cannot assume that this would be so if the agent were administered at more frequent intervals. These studies on experimental infections in monkeys raise further questions concerning the variations that may be encountered in predictability of comparative in vivo efficacy of antimicrobials based on in vitro data.

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