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Comparative Efficacy of Cephalexin and Ampicillin for Shigellosis and Other Types of Acute Diarrhea in Infants and Children

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Most ampicillin-resistant *Shigella* are susceptible to cephalexin. Randomized treatment with cephalexin or ampicillin was given to 154 infants and children with acute diarrhea. Rectal swab cultures revealed *Shigella* in 42%, *Salmonella* in 6%, enteropathogenic *Escherichia coli* in 2%, and no pathogen in 50%. Cephalexin failed to eradicate *Shigella* after 5 days of treatment in 76% of patients as contrasted with 28% of ampicillin-treated patients with susceptible organisms. *Shigella* persisted in 78% of ampicillin-treated patients with resistant organisms. Diarrhea lasted more than 5 days in 43% of cephalexin-treated patients, in 56% of the ampicillin group with resistant organisms, but in only 9% of ampicillin-treated patients with susceptible organisms. The failure of cephalexin was due to the relatively high minimal inhibitory concentrations and minimal bacterial concentrations of 5 or 10 μg/ml and, although serum concentrations were twice the minimal bacterial concentration, they were not sufficient to demonstrate killing by the serum dilution method. In vitro susceptibility or resistance of *Shigella* to ampicillin correlated with clinical success or failure. Cephalexin is not a suitable drug for treatment of shigellosis in patients with ampicillin-resistant organisms.

Considering the rapidly increasing problem with ampicillin resistance of *Shigella sonnei* strains in the United States (2) it becomes important to look for suitable alternative drugs for treatment of severe shigellosis. Shigella strains that are multiply resistant to sulfas, tetracycline, chloramphenicol, and ampicillin generally retain their in vitro susceptibility to cephalosporin derivatives. In a previous study (5) we found that only one of 160 shigella strains was resistant to cephalothin, cephaloridine, cephaloglycin, and cephalexin. Cephaloglycin proved ineffective for shigellosis, presumably on the basis of the poor absorption, with most patients having no measurable levels of cephaloglycin in their blood. It therefore seemed worthwhile to carry out a clinical trial with cephalexin, which has much better absorption than cephaloglycin and quite consistently gives peak serum levels over 10 μg/ml with the recommended dosages.

MATERIALS AND METHODS

This study was done in the outpatient clinic of Children's Medical Center in Dallas and the protocol was essentially the same as in our previous study of ambulatory patients with diarrhea comparing ampicillin and placebo (3). Infants and children with acute diarrhea were enrolled in the study at the initial clinic visit. Patients with respiratory infections or other possible causes of nonspecific diarrhea were excluded. They were assigned from random numbers lists to treatment either with cephalexin in a daily dosage of 80 mg/kg or with ampicillin in a daily dosage of 100 mg/kg. Both drugs were given in four divided doses for 5 days. When more than one child in a family was studied, all received the same drug to avoid possible confusion of medications.

Rectal swab cultures were examined by standard bacteriological methods (1) by using eosin-methylene blue agar, xylose-lysine-desoxycholate agar, tergitol-7 with tetrazolium chloride, and enrichment in tetrathionate broth (with 1:100,000 brilliant green and 2% iodine solution) for subculture to brilliant green agar.

Shigella and *Salmonella* isolates were submitted to the Texas State Department of Health Laboratory for confirmation and serotyping.

In vitro susceptibility testing of pathogens was performed by the broth dilution method using ampicillin and cephalexin laboratory standards. The inoculum was 10^5 bacteria. Twofold dilutions of antibiotic from 1,280 μg/ml to 0.3 μg/ml were made in sensitivity test broth. After incubation at 37 C for 18 h the minimal inhibitory concentration was noted and tubes without visible turbidity were subcultured to 5% sheep's blood agar to determine the minimal bactericidal concentration.

Measurements of cephalexin or ampicillin in serum

[1] Died 21 December 1973.

were performed by the micromethod of Simon and Yin (7) using finger-stick blood.

Serum inhibitory and bactericidal titers were done using twofold dilutions of serum in brain-heart infusion broth. The *Shigella* strain isolated from the patient was inoculated into each well in a microtiter plate (10^5 bacteria) and the cultures were incubated for 18 h at 37 C. Clear wells were subcultured to 5% sheep's blood agar to determine the bactericidal titer.

Written, informed consent was obtained from parents.

Patients returned for follow-up on days 2 or 3 and days 4 to 6 of treatment. Attempts were made to follow all patients with pathogens for 1 month at weekly intervals.

At each examination the interval history was recorded and rectal swab cultures were obtained. Mothers kept a daily record of the number and character of stools. Cessation of diarrhea was defined as the first day on which the patient had normal, formed stools. Compliance was evaluated by noting the volume of medication remaining in the bottle. Details of the clinical and bacteriological evaluations have been presented previously (3).

RESULTS

One hundred fifty-four infants and children were studied. *Shigellae* were identified in 64 patients (42%), and 77 patients (50%) had no recognized pathogen (Table 1). Ten patients had *Salmonella* infection and an additional patient had *Salmonella* and *Shigella* isolated from the initial rectal swab culture. Five infants had enteropathogenic serotypes of *Escherichia coli*, including two babies who also had *S. sonnei* in the first culture specimen. The distribution of patients in the ampicillin and cephalexin groups according to bacterial etiology was similar as was the distribution by *Shigella* serotypes (Table 2). Approximately two-thirds had *S. sonnei* infection in each group.

Patients in the two treatment groups were comparable with regard to age, sex, race, and duration and severity of diarrhea before the first clinic visit. In the ampicillin-resistant, ampicillin-susceptible, and cephalexin groups, respectively, the average ages were 3.0, 3.3, and 2.5 years, the average durations of illness before therapy were 5.1, 3.4, and 3.5 days, and the average numbers of stools in the 24 h before starting treatment were 6.3, 6.2, and 7.1.

In selected patients serum concentrations of cephalexin and ampicillin were measured at the time of the initial visit (Table 3). The first dose of medication was given by the research nurse and serum specimens were collected 1 or 2 h later. An attempt was made to get sequential serum specimens for antibiotic assay by having the mothers remain in the clinic with the children for 4 h, but only a small number of patients cooperated. The peak serum level occurred after 0.5 to 1 h with both drugs. The mean peak concentration of ampicillin was approximately one-fourth that of cephalexin, and the range of values was much greater for ampicillin. The marked variability of ampicillin absorption in patients with shigellosis has been documented by us previously (6).

All *Shigella* strains from cephalexin-treated patients were susceptible in vitro to cephalexin (Fig. 1). Most (82%) were inhibited by 5 μg/ml or less but only 22% were killed at that concentration. All were killed at levels of 10 μg/ml.

Nine strains from the 36 ampicillin-treated patients (25%) were resistant in vitro to ampicillin. The resistant strains in this group were

TABLE 1. *Rectal swab culture results of 154 outpatients with acute diarrheal disease*

Culture	Total no. of patients	Ampicillin	Cephalexin
No pathogen	77 (50%)	36 (46%)	41 (54%)
Shigella sp.[a]	64 (42%)	36 (46%)	28 (37%)
Salmonella sp.	10 (6%)	5 (6%)	5 (7%)
Enteropathogenic *E. coli*	3 (2%)	1 (1%)	2 (3%)

[a] Including three patients with two pathogens: *S. sonnei* + *E. coli* 0128; *S. sonnei* + *E. coli* 055; *S. flexneri* + *Salmonella* Gr. B.

TABLE 2. *Distribution of Shigella serotypes*

Serotype	Ampicillin (36 patients)	Cephalexin (28 patients)
Shigella flexneri 1a	1	0
S. flexneri 2a	6	3
S. flexneri 2b	3	2
S. flexneri 3a	1	1
S. flexneri 3b	1	0
S. flexneri 4a	0	3
S. flexneri 6	0	1
Shigella sonnei	24 (67%)	18 (64%)

TABLE 3. *Antibiotic serum concentrations after doses of cephalexin (20 mg/kg) or ampicillin (25 mg/kg)*

Drug	Serum concn in μg/ml			
	0.5 h	1 h	2 h	4 h
Cephalexin				
Mean	22.9	18.8	8.7	2.3
Range	8.8–37.5	10.5–39.8	2.2–22.7	1.3–5.8
n	6	39	36	4
Ampicillin				
Mean	4.5	5.4	3.7	2.3
Range	2.3–8	0.8–22	0.4–13.5	0.4–3.7
n	8	40	36	4

all *S. sonnei*. Therefore, of the total 24 *S. sonnei* strains from this group, 37.5% were ampicillin resistant. All 12 *S. flexneri* strains were susceptible. Among the 18 cephalexin-treated patients, only one was resistant to ampicillin. Therefore, of the total 42 *S. sonnei* strains, 10 (24%) were ampicillin resistant. There was one cephalexin-treated patient with *S. flexneri* who had an ampicillin-resistant strain. Therefore, of the total 22 patients with *S. flexneri*, one (4.5%) was resistant in vitro. The resistant strains almost all required ampicillin concentrations of 1,280 or greater for inhibition. Most susceptible strains were inhibited and killed by concentrations of 1.25 to 2.5 or less. Three strains requiring 5 μg/ml for inhibition and killing were considered of intermediate susceptibility.

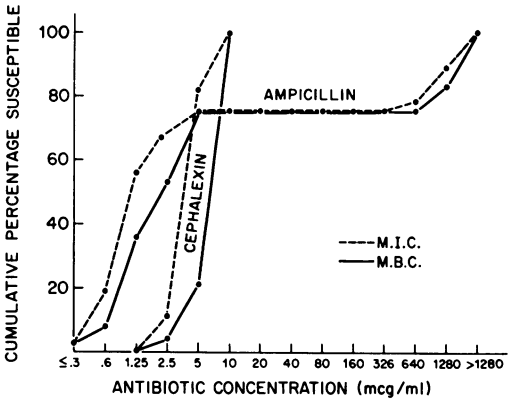

FIG. 1. *In vitro broth dilution susceptibilities of 36 Shigella strains to ampicillin and of 28 strains to cephalexin.*

The results of rectal swab cultures at follow-up visits from shigellosis patients are presented in Fig. 2. Responses of the ampicillin-treated group are divided into those nine patients with ampicillin-resistant organisms and the 27 patients with ampicillin-susceptible organisms. At the first follow-up examination 2 or 3 days after start of treatment, 88% of the cephalexin-treated patients and all nine ampicillin-treated patients with resistant organisms still had positive cultures compared with 22% of the ampicillin-treated patients with susceptible organisms. At the second follow-up 4 to 6 days after start of treatment, 76% of cephalexin-treated patients still had positive cultures, and 78% of the ampicillin treatment group with resistant organisms were positive. Specimens were obtained from only 67% of patients 10 to 20 days after start of treatment. The percentage of positive cultures increased in those who had been treated with ampicillin. This may have been reacquisition of infection in the environment or relapse. Reappearance of *Shigella* in the culture was not associated with return of diarrhea. In all cases the serotype of *S. flexneri* isolated at follow-up was the same as that isolated originally. Colicin typing was done on isolates from the patients with *S. sonnei* infection and in one case it clearly was a new infection because the patient had had colicin type 4 initially, but at follow-up had colicin

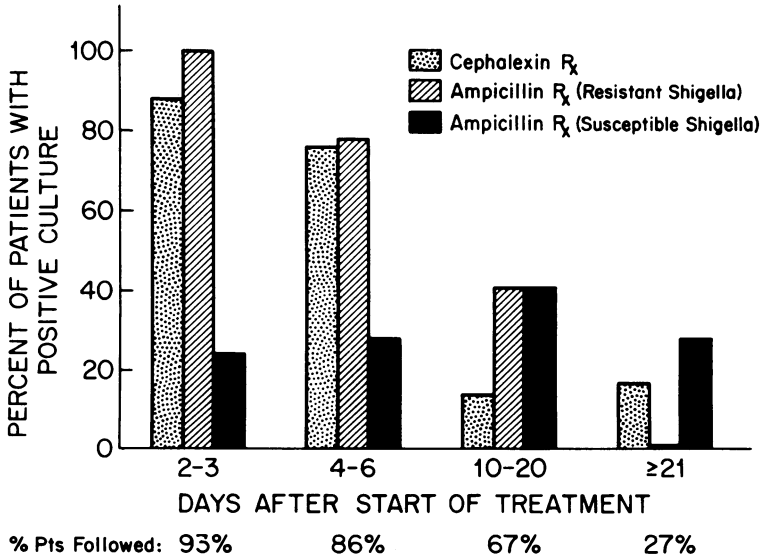

FIG. 2. *The frequencies of Shigella isolation from rectal swab cultures at follow-up examinations. The percentages of patients in each group followed at the four periods were as follows: cephalexin, 93%, 75%, 50%, 21%; ampicillin susceptible, 93%, 93%, 78%, 26%; ampicillin resistant, 100%, 100%, 78%, 22%.*

type 9. Approximately 20% of the patients had positive cultures after 21 days.

Diarrhea persisted longer than 5 days in approximately half of the shigellosis patients treated with cephalexin or those treated with ampicillin who had resistant organisms but in only 9% of the ampicillin-treated patients with susceptible organisms (Table 4). The average number of days after start of treatment until the diarrhea stopped was approximately twice as long in the cephalexin and ampicillin-resistant groups as in the ampicillin-treated patients with susceptible organisms. The average number of stools recorded by the mother was from 3.5 to 5.5 for the cephalexin- and ampicillin-resistant groups. After only 24 h of treatment the number of stools was reduced below three in the ampicillin group of patients with susceptible organisms.

Serum inhibition titers against the *Shigella* strain isolated from each patient are correlated with the measured antibiotic serum concentration in Fig. 3. In specimens from cephalexin-treated patients, with one exception, inhibition was demonstrated only at serum concentrations greater than 10 μg/ml, but of the total 20 specimens with concentrations greater than 10 μg/ml, only 7 (35%) demonstrated inhibition. By contrast, serum specimens with ampicillin concentrations of 2.5 μg/ml or greater were inhibitory to *Shigella* when the organisms were susceptible in vitro to less than 5 μg/ml with the exception of two patients with levels of 2.7 and 3.3 μg/ml. Organisms from three patients had minimal inhibitory concentrations of 5 μg/ml. Serum specimens with concentrations of 4, 4.3, and 4.7 μg/ml failed to demonstrate inhibition against these intermediate strains. No inhibitory titer was demonstrable in serum specimens from patients with ampicillin-resistant organisms. (The antibiotic concentrations were low in these patients because, by chance, they were 2-h specimens while most specimens from patients with susceptible organisms were obtained 1 h after a dose.)

Eradication of *Shigella* from the rectal swab cultures correlated well with presence or absence of a serum bactericidal titer (Fig. 4). In

TABLE 4. *Response of diarrhea to treatment in patients with shigellosis*

Determinants	Treatment groups		
	Cephalexin	Ampicillin (resistant *Shigella*)	Ampicillin (susceptible *Shigella*)
Patients with diarrhea >5 days after start of therapy	43%	56%	9%
Avg. days after start of treatment until diarrhea stopped	5.8	6.1	3.4
Avg. no. of stools daily after start of treatment			
Day 1	5.4	5.5	2.9
Day 2	5.2	3.3	2.8
Day 3	3.3	4.0	2.4
Day 4	3.5	4.0	2.1
Day 5	3.5	3.8	2.3

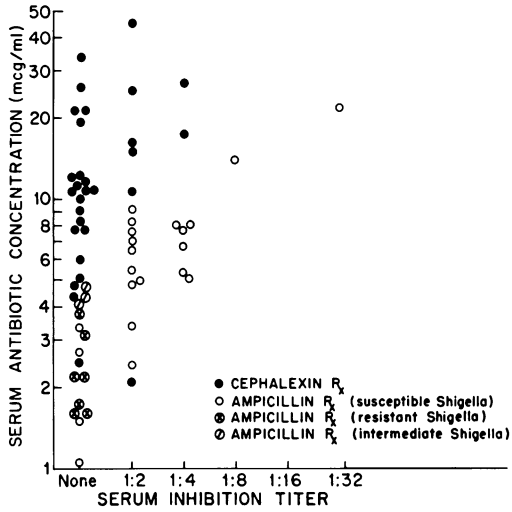

FIG. 3. *Comparison of serum inhibition titer against the Shigella isolated from each patient and the measured concentration of antibiotic in serum. The minimal inhibitory concentrations of susceptible Shigella were <5 μg/ml, of intermediate strains 5 μg/ml and of resistant organisms ≥ 10 μg/ml. All Shigella were susceptible in vitro to cephalexin.*

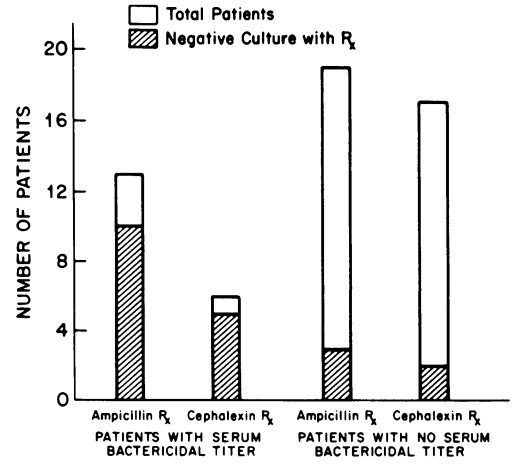

FIG. 4. *Relation of presence or absence of a serum bactericidal titer against Shigella to the suppression of Shigella in rectal swab cultures.*

the majority of patients in whom no serum bactericidal titer was demonstrable, *Shigella* persisted in the stool culture during treatment. By contrast, in most cases where a serum bactericidal titer was demonstrable, the cultures were negative during treatment.

The bacteriological and clinical responses of patients with shigellosis were analyzed by the chi-square test. Differences in frequency of positive rectal swab cultures on days 2 to 3 were significant between the ampicillin-susceptible group and the cephalexin group ($P < 0.0005$) and the ampicillin-resistant group ($P < 0.0005$), but not between the cephalexin group and the ampicillin-resistant group ($P < 0.30$). On days 4 to 6 the results were $P < 0.005$, $P < 0.01$, and $P < 0.90$, respectively, for the three comparisons. Differences between groups at the 10- to 20-day and later follow-ups were not statistically significant ($P < 0.30$ to $P < 0.90$). Persistence of diarrhea after 5 days treatment was significantly different between the ampicillin-susceptible group and cephalexin group ($P < 0.005$) and the ampicillin-resistant group ($P < 0.005$) but not between the cephalexin group and the ampicillin-resistant group ($P < 0.40$).

There were very few patients with *Salmonella* or enteropathogenic *E. coli* disease (Table 5). In the 11 patients with *Salmonella* the stool cultures of most remained positive during therapy with cephalexin or with ampicillin. Seven of the 11 patients still had positive cultures after 10 to 20 days. Only four were followed up longer than 21 days, and two of these remained positive. All five patients with enteropathogenic *E. coli* had negative cultures at the end of treatment. *Salmonella* patients treated with ampicillin had diarrhea persisting for an average of 5 days (range of 2 to 7 days) and of those treated with cephalexin it lasted for an average of 4.6 days (range of 3 to 6 days).

Of the patients without recognized pathogens at the beginning of treatment, 20% of those who received ampicillin and 11% of those who were treated with cephalexin still had diarrhea after 5 days of treatment. In the previous outpatient study (3) comparing ampicillin and placebo similar results were observed. Patients without pathogens had diarrhea after 5 days in 19% of ampicillin-treated patients and in 20% of placebo-treated patients. Thus, antibiotics appear to have no obvious beneficial or adverse effect in such patients when the drugs are given for only 5 days.

TABLE 5. *Bacteriological responses of patients with Salmonella or enteropathogenic E. coli diarrhea*

Organism and treatment	Days after start of treatment[a]			
	2-3	4-6	10-20	>21
Salmonella				
Cephalexin	3/6	4/6	2/6	2/2
Ampicillin	4/5	4/5	5/5	0/2
Enteropathogenic *E. coli*				
Cephalexin	1/3	0/3	0/3	
Ampicillin	0/2	0/2	0/2	

[a] No. with positive culture/no. studied.

DISCUSSION

The failure of cephalexin to eradicate *Shigella* and to shorten the period of diarrhea in most patients with shigellosis in this study appears to be due to the fact that sufficient concentrations in serum are not achieved. Shigella are not resistant to cephalexin by the usual standards of susceptibility and resistance but the minimal inhibitory and bactericidal concentrations of 5 or 10 μg/ml are relatively high. Although the mean peak serum concentration measured in these patients was almost 20 μg/ml and no patient had a peak level below 10 μg/ml, this concentration of cephalexin appears insufficient to inhibit and kill *Shigella* organisms by the serum dilution technique. This is further substantiated by the observation that the few patients treated with cephalexin who had negative cultures during treatment were the same few who demonstrated serum bactericidal titers in vitro.

This explanation of the failure of cephalexin is consistent with our previous conclusions that the most important determinant of success in treating shigellosis is attainment of effective serum, and presumably tissue, concentrations of antibiotic. The extreme variability of serum concentrations after orally administered ampicillin was once again documented in this study. This had previously been shown in hospitalized children with more severe shigellosis (6) and the children with poor absorption maintain positive stool cultures and have persistent diarrhea longer than those who achieve higher serum levels of ampicillin (4). Undoubtedly this variable absorption of ampicillin accounts for some therapeutic failures in patients with shigellosis due to ampicillin-susceptible organisms.

We have not previously been able to treat a large enough group of patients with ampicillin-resistant organisms with ampicillin to prove that therapeutic success or failure correlates with in vitro susceptibility or resistance but the differences were statistically significant in this study.

It is concluded that cephalexin is not a suitable alternative drug for treatment of shigellosis in patients with ampicillin. Presumably, if the drug were given in a larger dosage, effective suppression of *Shigella* would result. The manufacturer's recommended dosage is 25 to 50 mg/kg per day and it is doubtful that patients would tolerate dosages much larger than the 80 mg/kg per day used in this study.

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