Oxolinic Acid in the Treatment of Typhoid Fever Due to Chloramphenicol-Resistant Strains of *Salmonella typhi*

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Of 28 strains of *Salmonella typhosa* collected in late 1972 in Vietnam, 4 had minimum inhibitory concentrations to chloramphenicol of >100 μg/ml. Median minimum inhibitory concentrations of all strains to oxolinic acid were 0.39 μg/ml; ampicillin, 6.25 μg/ml; amoxicillin, 0.39 μg/ml. Widespread typhoid fever appeared in mid-1973 and reached 78% in March 1974 and 91% in March 1975. In July 1974, a pilot study was begun to evaluate the efficacy of oxolinic acid in vivo, recognizing the discrepancy between in vitro and in vivo results with many agents evaluated in the treatment of typhoid fever. Seven patients with typhoid fever, six with positive blood cultures, were treated with oxolinic acid (1.5 g twice daily by mouth, a daily dose that averaged 75 mg/kg per day) for 5 to 12 days. In five of six patients, blood cultures became negative at 2 to 3 days, with another being negative at 6 days. Despite negative blood cultures, all but one patient remained clinically ill with temperatures of >39.5°C at 4 to 9 days. All strains were susceptible to 0.19 μg of oxolinic acid per ml, and resistant strains did not occur. One patient died after being changed to ampicillin, one left against advice, three responded to amoxicillin, and one died with pseudomonas bacteremia. Toxicity to oxolinic acid did not occur.

Chloramphenicol has been the cornerstone of treatment of typhoid fever since 1948 (21). In 1952 Woodward and associates pointed out the lack of therapeutic responses in patients treated with penicillin, streptomycin, polymyxin B, chlorotetracycline, and oxytetracycline, although each of these drugs appeared to be more effective than chloramphenicol in vitro (22). Beginning in 1962, initially as isolated instances, strains of *Salmonella typhi* which were resistant to chloramphenicol began to be reported (9). In September 1971, chloramphenicol-resistant strains of *S. typhi* were isolated from patients in the Saigon area of Vietnam (2). The proportion of resistant strains increased progressively, being 14% in the fall of 1972, 25 to 78% in 1973, and reaching 91% in March 1974 (6, 12-14, 18). Late in 1973 an increasing number of patients with typhoid fever began being admitted to hospitals in Saigon.

Since the quinoline carboxylic acid, oxolinic acid, had been shown to be effective against several strains of *S. typhi* in vitro (19), the present study to evaluate the efficacy of oxolinic acid in vitro against strains of *S. typhi* and subsequently its effectiveness in patients with typhoid fever was initiated.

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MATERIALS AND METHODS

Bacterial cultures. Subcultures of 28 strains of *S. typhi* that had been isolated originally in the fall of 1972 were kindly provided by E. R. Rhode of the University of Oklahoma Medical Center (2).

Antimicrobial agents. Oxolinic acid was obtained as the powder (lot 28) and as 2-μg disks (lot 4) from the Warner-Lambert Research Institute. Amoxicillin was obtained as the powder and as 10-μg disks from Beecham, Inc. Chloramphenicol was obtained from Parke, Davis & Co. for susceptibility testing. Sterile sodium ampicillin and tetracycline hydrochloride were obtained from the hospital pharmacy.

Susceptibility testing. (i) Agar dilution method. Round petri dishes (100 by 15 mm) were each filled with 20 ml of Mueller-Hinton agar (Difco) containing in twofold dilutions 0.09 to 12.5 μg of oxolinic acid per ml, 0.09 to 3.12 μg of amoxicillin per ml, and 1.56 to 100 μg of chloramphenicol, ampicillin, and tetracycline per ml. Plates were stored at 4°C.
but used within 1 day of preparation. Individual strains were grown overnight in 5.0 ml of Trypsinase soy broth (BBL) at 37 C, with dilution to 1:100 in sterile distilled water prior to inoculation onto drug-containing plates with a Steers replicating device (17). Plates were incubated at 37 C overnight. The minimal inhibitory concentration (MIC) was read as the least concentration of antibiotic that inhibited visible growth. A barely visible haze of growth or up to five colonies was interpreted as "inhibition."

(ii) Antimicrobial disc agar diffusion method. Susceptibility tests were performed by the Bauer-Kirby method (1). The disk potency and zone size interpretive criteria were those that are standard (1) for chloramphenicol, ampicillin, and tetracycline. For oxolinic acid (2-µg disks), zone diameters of 19 mm or greater were considered as susceptible.

Clinical studies. All patients were hospitalized on the Infectious Diseases Service of Cho Quan Hospital between 3 and 24 July 1974 and were seen by one of us (JPS). Eligibility for entrance into the study required an initial clinical impression of typhoid fever, negative peripheral blood smears for plasmodia, ability to take medications orally, and informed consent on the part of the patient and family. The study was explained to the patients in Vietnamese by one of us (NNL) or by a staff physician. Initial clinical and laboratory studies included at least a complete physical examination, hematocrit, leucocyte count with differential, urinalysis, blood urea nitrogen, venous blood culture, Widal test, and stool culture. Follow-up evaluation included at least daily examination by one of us including careful attention to side effects (JPS), temperatures at least twice daily, and repeat blood cultures on day 3, day 5 to 7, and at the completion of therapy (day 10). All data were recorded on standardized forms.

The initial trial design was to evaluate five patients in a nonrandom consecutive case study. Patients received two 750-mg tablets of oxolinic acid every 12 h by mouth (approximately 75 mg/kg per day). If therapy was ineffective as based upon clinical deterioration, occurrence of complications, failure of temperature response within 7 days, or positive blood cultures after day 5 of treatment, the patient was switched to an alternative agent, amoxicillin, ampicillin, or trimethoprim-sulfamethoxazole.

RESULTS

In vitro susceptibility of strains of *S. typhi*. The susceptibilities of the 28 strains isolated in 1972 are presented in Fig. 1. The median MIC for both oxolinic acid and amoxicillin was 0.39 µg/ml with all 28 strains inhibited by 0.78 µg or less of oxolinic acid per ml and 1.56 µg or less of amoxicillin per ml. Ampicillin was less active than amoxicillin, with the median MIC for ampicillin being 3.12 µg/ml. One isolate was not inhibited by ampicillin at a concentration of 100 µg/ml. The median MIC for chloramphenicol was 6.25 µg/ml; however, four isolates (14%) were not inhibited by 100 µg/ml. These four isolates were also resistant to tetracycline, but all were susceptible to oxolinic acid and amoxicillin at concentrations of 0.39 µg/ml. The single ampicillin-resistant isolate was susceptible to chloramphenicol at 25 µg/ml.

Clinical characteristics and results of treatment with oxolinic acid. Seven patients, ages 13 to 28 years, three males and four females, were entered into the study between 5 and 10 July 1974 (3 on July 5, 1 on July 6, and 3 on July 10) (Table 1). Each of the patients had fever.

![Fig. 1. Cumulative percentage of 28 strains of *S. typhi* isolated in 1972 inhibited by various concentrations of antimicrobial agents (agar dilution method).](http://aac.asm.org/Downloaded_from http://aac.asm.org on November 3, 2017 by guest)
Three had abdominal pain, three were constipated, and three had diarrhea. Other symptoms included headache in four patients, cough in four, and epistaxis in one. Six of the seven patients had received injections and/or tablets of medication for the admitting illness prior to hospitalization. The only patient in whom the medication was known (no. 4) had received 5.0 g of ampicillin in the preceding week. In the other patient the medications were unknown. One patient (no. 3) had received antityphoid immunizations; he had received his last injection in 1970. Only one patient (no. 7) had underlying medical problems, she being a morphine addict. All of the patients, except no. 7, were lucid; no. 7 was intermittently delirious. On initial examination, two patients had relative bradycardia, two had wheezing on auscultation of the lungs, four had minimally palpable spleens (1 to 2 cm), and three had rose spots. Total leukocyte counts ranged from 3,600 to 6,850/mm³. Initial blood cultures were positive in six of the seven patients (Table 1).

Patient 1 clinically had typhoid fever, came from the area near Saigon where the prevalence of typhoid was highest, and had been ill for 1 month prior to hospitalization, during which time he had received unidentified injections for 6 or 7 days and tablets. His Widal agglutination revealed a typhoid O titer of 1:320 (no history of immunization). Based upon these findings, he was considered to have probable typhoid fever. Clinically he improved within 48 h of starting oxolinic acid, becoming afebrile by day 3 (July 8). Based upon his response, the last three patients were admitted into the study.

Each of the remaining six patients had positive blood cultures. Patients 2 to 6 had definite typhoid fever. The diagnosis in patient 7 is less secure. Two pretreatment blood cultures that had been drawn separately were reported as positive for S. typhi, and one was lost on subculture; the other subculture upon return to Dallas grew only Pseudomonas aeruginosa. Hence, although this patient is included as typhoid fever, the diagnosis can be questioned. While on oxolinic acid, none of the remaining six patients showed consistent subjective improvement, although on occasion patients 3 and 6 said they felt much improved. Each of the patients remained febrile with temperatures of 38.5 to 40.5 C after 5 to 9 days of oxolinic acid (Table 1). Patient 6 had clinically improved but was still febrile and left against medical advice on July 15. Based upon these failures to respond clinically, on July 15 to 16 each of the patients was changed to an alternative agent; four were begun on 1.0 g of amoxicillin 3 times daily by mouth and one on 3.0 g of ampicillin daily intravenously.

Complications included the development of melena and significant gastrointestinal bleeding in two patients. Patient 2 developed melena on the day he was changed to amoxicillin. Patient 5 developed melena 2 days after admission, with a drop in hematocrit from 39 to 32%, which then remained stable. Because of failure to defervesce, she was begun on intravenous ampicillin. The next day she had massive hematochezia; the shock responded to transfusion; however, the next day she died, without evidence of further bleeding. Patient 7 was found dead during the night 2 days after being changed to amoxicillin. The final event could
not be ascertained although she was a known morphine addict and in retrospect may well have had pseudomonas bacteremia rather than typhoid fever.

No side effects attributable to oxolinic acid were observed although each patient was specifically questioned each day for the development of nausea, vomiting, insomnia, and restlessness (the more common adverse effects encountered in patients receiving oxolinic acid for the treatment of urinary tract infections). The three patients (no. 2, 3, 4) who completed reasonable courses of amoxicillin became afebrile (temperature, <37.8°C) within 3 to 5 days (no. 2 on day 5, no. 3 on day 3, no. 4 on day 5). Patient 2 developed a generalized maculopapular skin rash on day 10 of amoxicillin.

Although oxolinic acid was not clinically effective, in four of the five patients with definite typhoid fever whose initial blood cultures were positive for S. typhi, blood cultures repeated 2 to 3 days after beginning oxolinic acid showed no growth (Table 2). In vitro susceptibilities performed in Saigon by using the disc agar diffusion method revealed each of the initial isolates to be resistant to chloramphenicol. Subcultures were transported to Dallas and were studied by the agar dilution method for susceptibility to oxolinic acid, chloramphenicol, ampicillin, and amoxicillin (Table 2). Of the four subcultures of initial isolates, each was susceptible to oxolinic acid at 0.19 μg/ml; three of the four were resistant to chloramphenicol at greater than 100 μg/ml, whereas each was susceptible to ampicillin and amoxicillin. In the one patient (no. 5), in whom posttreatment cultures were positive for S. typhi at 2 and 5 days, the subculture of the isolate from day 2 revealed no change in susceptibility to oxolinic acid. Unfortunately, the subculture of the isolate from day 5 was lost.

**DISCUSSION**

The occurrence of the epidemic of typhoid fever caused by chloramphenicol-resistant S. typhi in Mexico City in 1972 raised questions such as the usefulness of the in vitro observations as correlates of in vivo responsiveness and alternative agents that would be effective if chloramphenicol were not.

In Mexico City, many physicians continued to use chloramphenicol, despite in vitro resistance. In controlled studies 15 patients were treated with intravenous chloramphenicol (of assayed potency) at a dosage of 100 mg/kg (5). Thirteen of these patients were infected with

**Table 2. Results of blood cultures and in vitro susceptibilities with chloramphenicol-resistant typhoid fever in patients treated with oxolinic acid**

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<tr>
<th>Patient</th>
<th>Blood culture</th>
<th>Antimicrobial susceptibilities MIC (μg/ml)</th>
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*Abbreviations: OA, oxolinic acid; CM, chloramphenicol; AMP, ampicillin; AMOX, amoxicillin; (R), resistant by disc agar diffusion method.  
* Days after beginning oxolinic acid.
chloramphenicol-resistant \textit{S. typhi} (MIC, $>50\, \mu g/ml$). Of these 15 patients, 3 required a change in therapy, 3 developed complications, and 11 who responded initially relapsed and required retreatment. As late as day 5, blood cultures remained positive in 6 of 10 patients on chloramphenicol, whereas none of 9 was positive in the patients who received ampicillin. Thus, there appeared to be good correlation between the lack of in vitro susceptibility to chloramphenicol and failure of clinical response.

After the initial appearance of chloramphenicol-resistant strains of \textit{S. typhi} in South Vietnam in September 1971, by March 1974 over 90\% of the strains of \textit{S. typhi} were resistant. These strains also were resistant to tetracycline, streptomycin, and sulfadiazine (3). Resistance was associated with three Vi phage types and was transferable to recipient Escherichia coli K-12 (3). In addition, strains that were resistant to ampicillin were noted (13). The number of definite cases of typhoid at one hospital in the area of Saigon (Nguyen Van Hoc, Gia Dinh) increased from 7 to 9 patients per month in late 1973 to 80 cases in April 1974 (18). During the present study (3 to 24 July 1974), 22 patients with typhoid fever were admitted to the infectious disease wards of another Saigon Hospital (Cho Quan Hospital).

In consideration of alternatives to chloramphenicol, it has long been recognized that a major dichotomy may exist between the in vitro susceptibility of \textit{S. typhi} to an antimicrobial agent and the efficacy of the agent in patients (20). The major alternative agents that have been demonstrated to be effective in patients are ampicillin, amoxicillin, and trimethoprim-sulfamethoxazole (2, 4, 5, 7, 10, 12, 15, 18). However, ampicillin has not been as effective as chloramphenicol and, in addition, is more expensive, hence often limiting its availability, especially in areas of need (7, 12).

It is against this background that the present studies of oxolinic acid were performed. Oxolinic acid is a synthetic quinoline carboxylic acid which is structurally related to nalidixic acid but which is considerably more active (approximately 30 times) than nalidixic acid (16, 19). Initial antimicrobial screening included two strains of \textit{S. typhi}, both of which were inhibited at 0.78 $\mu g$ of oxolinic acid per ml (19). In humans, mean peak serum concentrations of oxolinic acid were $3\, \mu g/ml$ after a 1,000-mg oral dose (N. H. Schimmel, Warner-Lambert Research Institute, personal communication). The administration of oxolinic acid (1.5 g/day) to volunteers with experimental shigellosis revealed no clinical failures and a bacteriological response in 14 of 20 subjects, and it has been suggested as a potential alternative agent to ampicillin (8).

The in vitro studies reported herein confirm the susceptibility of both chloramphenicol-susceptible and -resistant strains to oxolinic acid. Based upon these in vitro observations, the effectiveness of oxolinic acid in shigellosis, a disease that requires systemic absorption of a drug (8, 11), its potentially lower cost, and relative freedom from adverse effects other than those of a nuisance nature, a pilot clinical study was performed. Unfortunately, oxolinic acid is another agent that can be added to the list of antimicrobials to which \textit{S. typhi} strains are susceptible in vitro but which are ineffective in vivo.

These studies illustrate another rather unique enigma in the saga of the treatment of typhoid fever. Calderon and associates observed in a group of patients treated with ampicillin that blood cultures were converted to negative within 2 days; yet the mean duration of fever was 5.5 days (5). Although our patients failed to respond clinically, blood cultures became negative within 3 days in four of five patients.

Finally, these observations add limited support to the studies on the effectiveness of amoxicillin in the treatment of typhoid fever caused by chloramphenicol-resistant \textit{S. typhi} (4).

\textbf{ACKNOWLEDGMENTS}

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\textbf{LITERATURE CITED}


