In Vitro Activity of MK-0366 Against Clinical Urinary Pathogens Including Gentamicin-Resistant *Pseudomonas aeruginosa*

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MK-0366, a new derivative of nalidixic acid, was tested against 250 urinary pathogens including *Escherichia coli*, *Serratia marcescens*, and *Pseudomonas aeruginosa*. This new agent was more active than any of the other antibiotics tested, which included carbenicillin, ampicillin, cephalaxin, tetracycline, trimethoprim, trimethoprim-sulfamethoxazole, and nalidixic acid. Gentamicin-resistant *P. aeruginosa* were highly sensitive to MK-0366, with a 90% minimal inhibitory concentration of 0.8 μg/ml. *Serratia* strains were the most resistant organisms, with a 90% minimal inhibitory concentration of 3.1 μg/ml. These results suggest that clinical trials should be designed to investigate the clinical usefulness of this new drug in urinary infections.

Nalidixic acid analogues synthesized recently, such as pipemidic acid and others, have exhibited significant antimicrobial activities toward bacteria that are beyond the spectrum of nalidixic acid (2, 5; Y. Nishimura, H. Kishi, O. Tsukada, T. Tominaga, T. Niijima, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother., 20th, New Orleans, La., abstr. no. 76, 1980; J. Shimada, Y. Ueda, and T. Yamazi, 20th ICAAC, abstr. no. 75). One of these compounds, 1-ethyl-6-fluoro-1,4 dihydro-4-oxo-7-(1-piperazynyl)-3-quinoline carboxylic acid (MK-0366), has been found to have superior inhibitory activity for *Pseudomonas* and *Serratia* species (2). MK-0366 has also been shown to be very active against *Neisseria gonorrhoeae* (4).

The purpose of this study was (i) to report the in vitro activity of MK-0366 against 250 clinical urinary isolates, (ii) to compare the activity of MK-0366 with other oral antimicrobial agents in common clinical use, and (iii) to assess the potency of MK-0366 against gentamicin-resistant *Pseudomonas aeruginosa*. Specifically, the in vitro activity of MK-0366 was compared with the activities of cephalaxin, carbenicillin, tetracycline, trimethoprim, trimethoprim-sulfamethoxazole, and nalidixic acid against *P. aeruginosa*, *Escherichia coli*, and *Serratia marcescens* isolates.

Laboratory standards of the eight antibacterial agents tested were kindly supplied by their manufacturers. Each drug was tested over 11 twofold serial dilutions from the highest concentration: ampicillin, cephalaxin, and nalidixic acid, 200 μg/ml; tetracycline and trimethoprim, 100 μg/ml; MK-0366, 12.5 μg/ml; and trimethoprim-sulfamethoxazole 100 μg/500 μg.

We used 100 strains of *E. coli*, 100 strains of *P. aeruginosa*, and 50 strains of *S. marcescens*. These organisms were urinary isolates processed within the past 3 years by the clinical microbiology laboratories of the Yale-New Haven Hospital, New Haven, Conn., and the West Haven Veterans Medical Center, West Haven, Conn. Of the *Pseudomonas* isolates, 44 were characterized by the Veterans Administration Medical Center laboratory as carbenicillin- and gentamicin-resistant by the standard Kirby-Bauer disk test. They were resistant uniformly to carbenicillin, gentamicin, and all other antibiotics routinely tested. A few of these strains were sensitive to tobramycin, and most were sensitive to amikacin. Mueller-Hinton agar and Mueller-Hinton broth were obtained from BBL Microbiology Systems, Cockeysville, Md. Single batch-lot numbers of each medium were used. Mueller-Hinton broth was used without supplementary divalent cations.

Minimal inhibitory concentrations (MICs) were determined as previously described (1). Twofold serial dilutions of each drug were mixed in Mueller-Hinton broth, and 0.1 ml of each dilution was delivered to a standard 96-well microtiter plate. Organisms were grown in Mueller-Hinton broth, and plates were inoculated to a final concentration of 10⁶ colonies per ml (10⁴ colony-forming units per well). MICs were scored after an 18- to 20-h incubation at 37°C as the lowest concentration (well) showing no visi-
FIG. 1. Cumulative percentage of (A) 100 strains of *E. coli*, (B) 100 strains of *P. aeruginosa*, and (C) 50 strains of *S. marcescens* inhibited by increasing concentrations of MK-0366 (X-X), ampicillin (AMP, ●●), cephalexin (CXN, ■■), nalidixic acid (NA, □□), tetracycline (TETRA, △△), trimethoprim (TRIM, ○○), and trimethoprim-sulfamethoxazole (TMX, ▲▲).
bles growth. Each row of drug dilutions included a drug-free well as a viability and inoculation control.

After microtiter plates were scored for MICs, 1.5 μl from each well in each plate was transferred to drug-free Mueller-Hinton agar incubated at 37°C for 18 h, and scored as the lowest concentration of drug showing no colonial growth.

The activity of MK-0366 exceeded the activities of all of the antimicrobial agents tested against the 100 clinical isolates of E. coli (Fig. 1A). Most of the organisms were sensitive to readily attainable levels of nalidixic acid, cephalaxin, ampicillin, and tetracycline. The activities of trimethoprim and trimethoprim-sulfamethoxazole were significantly lower. MK-0366 was potent at levels nearly 100-fold lower than were the other antimicrobial agents with 50% MIC (MIC₅₀) values of 0.05 μg/ml and MIC₉₀ values of 0.2 μg/ml.

P. aeruginosa isolates were uniformly resistant to nalidixic acid, ampicillin, and cephalaxin (Fig. 1B). Tetracycline and trimethoprim-sulfamethoxazole had MIC₉₀ values of 50 μg/ml, whereas trimethoprim had an MIC₉₀ of 100 μg/ml. Carbencillin sensitivities reflected the Kirby-Bauer results, with one group (56 strains) sensitive with MIC₉₀ values of 25 μg/ml, whereas the remainder (44 strains) were more resistant with MIC₉₀ values of 200 μg/ml. MK-0366 was as active against both groups of Pseudomonas strains as it had been against E. coli, with an MIC₅₀ of 0.1 μg/ml and an MIC₉₀ of 0.8 μg/ml.

MK-0366 was least effective against S. marcescens strains, with an MIC₅₀ of 0.4 and an MIC₉₀ of 3.1 μg/ml. This, however, was more than 10 times lower than MICs obtained for any of the other antimicrobial agents tested (Fig. 1C). MIC₉₀ values for ampicillin and cephalaxin were greater than the highest concentrations tested (200 μg/ml). The MIC₉₀ values for trimethoprim-sulfamethoxazole and trimethoprim were greater than 100 μg/ml.

In only one instance was there a difference of more than twofold between MIC and minimal bactericidal concentration values. This was the activity of tetracycline against Pseudomonas strains, in which the MIC₅₀ was 6.3 and the 50% minimal bactericidal concentration was 50 μg/ml. Many of the 90% minimal bactericidal concentration values exceeded the highest concentration of drugs tested. The minimal bactericidal concentration for trimethoprim-sulfamethoxazole exceeded the maximum level tested for all 250 strains. MK-0366 was active at very low concentrations against more than 90% of all of the organisms tested, including gentamicin-resistant P. aeruginosa.

MK-0366 is a recently synthesized quinoline-carboxylic acid which has antibacterial activity against a wider variety of bacteria, gram negative and gram positive, than does nalidixic acid (2). The present study indicates the striking efficiency of MK-0366 in killing Pseudomonas, Serratia, and Escherichia strains compared with the efficiency of other commonly used oral antimicrobial agents. We did not compare the new drug with parenteral antimicrobial agents. Of particular interest is the high potency of MK-0366 for organisms (Pseudomonas and Serratia strains) which often colonize patients subsequent to immunosuppression or broad-spectrum antimicrobial therapy.

The 50 Serratia strains proved most resistant to MK-0366. At the 90% inhibitory level, MK-0366 and nalidixic acid, the only drugs effective against these 50 strains, showed a 32-fold difference in concentration (3.1 and 100 μg/ml, respectively). Similarly, MK-0366 was the only drug active against 90% of the Pseudomonas strains at easily achievable concentrations. This is of particular importance for the carbencillin- and gentamicin-resistant strains. Carbencillin-resistant Pseudomonas urinary tract infections are frequently treated with parenteral aminoglycosides in clinical practice. MK-0366 may represent a therapeutic alternative.

Whether MK-0366 will represent a valuable addition to the current antimicrobial agents used will require clinical trials. There is some evidence that it is effective in vivo as well as in vitro (3; Nishimura et al., 20th ICAAC, abstr. no. 75). These data suggest that further clinical trials should be undertaken to define the potential role of this new chemotherapeutic agent.

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LITERATURE CITED


