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Antipseudomonal Activity and Nephrotoxicity of Cephradine-Netilmicin Combination

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Abstract □ The effects of intraperitoneal injectiosn of cephradine in a dose of 75 mg/kg and netilmicin in dose of 50 mg/kg and their combination on creatinine and urea serum levels of rabbits were studied as well as the antipseudomonal activity against three multiresistant clinicial isolates. The antibacterial activity was investigated by two methods: Checkerboard titration method and time-kill studies. Finally, the antibacterial activity of the sera obtained from the rabbits receiving the used drugs in the previous regimen was studied using time-kill study method against *Pseudomonas aeruginosa* isolates. Results obtained from this study indicated that both creatinine and urea serum levels of the rabbits receiving both drugs were not significantly different from those of the rabbits receiving either cephradine or netilmicin alone. At the same time the *in vitro* antibactrial activity (either of the prepared solutions of the used drugs and their combination or of the sera obtained from the rabbits receiving these drugs as mentioned before) showed a synergistic effect against the tested strains of *Pseudomonas aeruginosa*

Keywords □ Antimicrobial activity, nephrotoxicity, *Pseudomonas aeruginosa,* cephradine, nitromicin, synergism

Antibiotic therapy of serious pseudomonas infections still presents a problem despite the introduction of several new effective antibiotics. In an effort to increase the effectiveness of the antibactrial therapy, many investigators have studied the effect of combination of two or more antibiotics on the efficacy of antibactrial therapy.[1,2] There are clinical situations where a combination of antibiotics is definitly indicated such as *Pseudomonas aeruginosa* infections. Cephalosporin-aminoglycoside combinations exhibited an effective antibiotic combination against such infections[3]. On the other hand, many case reports indicated that although antipseudomonal activity of many cephalosporin-aminoglycoside combinations was synergistic, the nephrotoxicity also was potentiated[3,4]. In an attempt to find out a cephalosporin-aminoglycoside combination of synergistic antibacterial activity and low nephrotoxicity, we decided to study the antipseudomonal activity and the nephrotoxicity of cephradine-netilmicin combination.

MATERIAL AND METHODS

In vitro studies.

Organisms: Three clinical isolates of *Pseudomonas aeruginosa* were obtained from the urine of three patients suffering from urinary tract infections, isolated and identified in the Department of Microbiology, Faculty of Pharmacy, Mansoura University.

Antibiotics: Cephradine (Velosef vials, Squibb) -Netilmicin (Nitromycin vials, Schering)

Media: Mueller-Hinton agar and broth (Difco) adjusted to pH 7.4 were used for culture and serial dilution.

Susceptibility test: The MICs for the three *Pseudomonas aeruginosa* clinical isolates were determined by the agar plate dilution method, with an inoculum of 10^4 cfu[5].

Studies of combined antimicrobial activity

Checker-board studies were performed with combinations of cephradine with netilmicin by the agar plate dilution method as previously described[1]. The fractional inhibitory concentration (FIG) was the minimum concentration of each of the two antibiotics that had an inhibitory effect when acting together[6]. The summation of the FICs of both antibiotics was the FIG index. The results were expressed as synergy, addition, indifference and antagonism when the values of the FIG index were <0.75, $0.75-<1.0$, $1.0-<2.0$, and >2.0 res-

pectively[6,7].

Killing curves

Killing curves of combined antibacterial activity were performed at least twice for the combination, following a slight modification of the method of Rosenblatt & Stewart[8] using 250 m*l* flasks[5]. For the combinations that showed synergy in the Checker-board studies, half the MIC was used in the flask with a single antibiotic, and one-eighth the MIC in the combinations of antimicrobial agents. When the combination showed indifference by the Checker-board, half the MIC was used alone and in combinations. For the antagonistic combinations in the Checker-board, the MIC was used alone in the combination[8].

All the flasks were incubated with shaking at 37° for 24 hours. At the start of the incubation, time 0 and after 1,2,4,8 and 24 hours of incubation, samples were taken of the culture in each flask. Colony counts were made on Muller-Hinton agar. The sensitivity of ten colonies to the two antibiotics was determined by agar dilution for detection of emergence of resistance.

With this method synergy was defined as a decrease of 2 $\log_{10}$ or more in the colony count when the antibiotic was used as compared with the count obtained with the most active single agent[1,9].

Antibacterial activity or cephradine and netilmicin in serum

Blood samples were collected after 6 hours of drug administration. Sera were separated by centrifugation and evaluated for antibactrial activity aganist *Pseudomonas aeruginosa* strain No. 2, using the time kill technique as described by Van Lathem *et al.*[10]. An overnight culture of the test strain of *Pseudomonas aeruginosa* was diluted in nutrient broth supplemented with calcium chloride (50 mg/*l*) and magnesium sulfate (20 mg/*l*) to yield 2×10^6 colony forming units per m*l*. To 1.5 m*l* portions of this preparation was added 0.5 m*l* of serum of rabbits received cephradine (9.5 mg/kg) or netilmicin (6 mg/kg) or their combination and then incubated at 37°C. Samples were removed at different time intervals for a period of 24 hours. Each sample was diluted in 10 fold steps in nutrient broth and plated on the surface of over dried nutrient agar medium. After 48 hours incubation at 37°C, the number of colonies on the plates was counted, the number of colony forming units/m*l* was calculated and the mean log number of colony-forming units of 6 values (representing the serum of six rabbits) was calculated.

Table I. Minimal inhibitory concentrations (MIC) against the strains of *Pseudomonas aeruginosa*

Strain No.	Antibiotic (μg/m*l*)	
	Cephradine	Netilmicin
1	31.25	16.00
2	62.50	16.00
3	125.00	32.00

In vivo studies

Drugs: Cephradine powder (Velosef, Squibb), prepared as 15% aqueous solution and injected *i.p.* in doses of 75 mg/kg (for nephrotoxicity studies) and 9.5 mg/kg (for combined antibactrial activity of rabbit serum).

-Netilmicin solution (Nitromycin vials, Schering), each 2 m*l* vial contains 150 mg netilmicin. It was injected *i.p.* in doses of 50 mg/kg (for nephrotoxicity studies) and 6 mg/kg (for combined antibactrial activity of rabbit serum).

Animals: Thirty rabbits of local strain each weighing 1.5-2 kg were used. They were classified into five groups each containing six rabbits. Group one received cephradine (75 mg/kg), group two received netilmicin (50 mg/kg), group three received combination of cephradine & netilmicin, group four received cephradine (9.5 mg/kg) and netilmicin (6 mg/kg), group five was used as control and received normal saline. All drugs were given once daily for 10 consecutive days.

Determination of serum urea and creatinine level: Twenty four hours after the last administration of the drugs blood samples were collected from the marginal ear vein, left to clot and centrifugated to separate the serum which was used for determination of the chosen parameters. Serum urea was measured by the enzymatic method described by Batton and Crouch.[11] Serum creatinine was measured by the method of Husdan[12].

RESULTS

Combination of Checker-board and killing curves results

In the killing curves only the results obtained after 8 and 24 hours incubation were considered in defining the interaction.

The combination of netilmicin with cephradine showed a very synergistic interaction by the Checker-board against *Pseudomonas aeruginosa* strain2

Table II. Results of the interactions in Checker-board and killing-curves

Pseudomonas aeruginosa strain No.	FIC index Checker-board	Killing curves 8 h.	Killing curves 24 h.
1	0.50	synergy	indifferent
2	0.18	synergy	synergy
3	0.53	indifferent	synergy

Synergy is defined as the reduction of 100 CFU/ml or more obtained with the combination compared with the combination as compared with the most active of the antibiotics alone used in the combination.

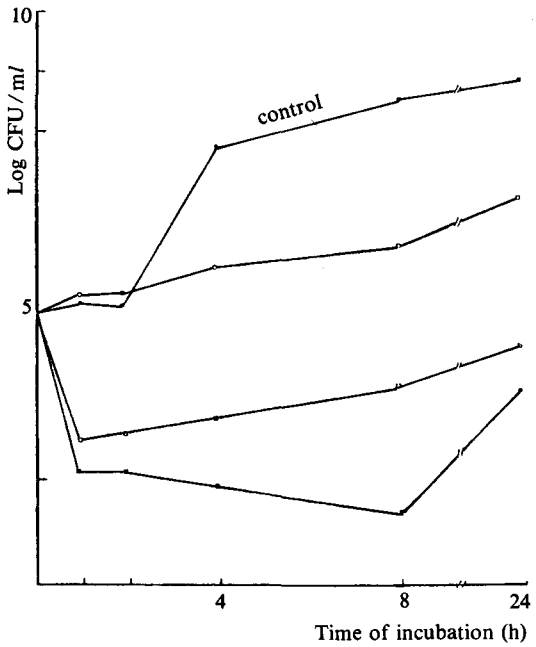

Fig. 1. Killing curves constructed from colony counts after incubation of *Pseudomonas aeruginosa* strain No. 1 with cephradine 15.62 μg/ml (○), nitelmicin 8.0 μg/ml (□) and cephradine-netilmicin 3.95 + 2.0 μg/ml (■) combination.

(FIC, 0.5, Table II). In the killing curves at 8 and 24 hours, the combination showed synergy (Fig. 2).

Against strain 1 the combination showed synergy in the Check-board (Table II) and in the killing curve at 8 hours, while at 24 hour showed re-growth (Fig. 1).

The combination against strain 3 showed synergy in the Checker-board (Table II). In the killing curves at 8 hours the action of the combination was equal to that of netilmicin alone, with re-growth in the flasks containing netilmicin at 24 hours, the

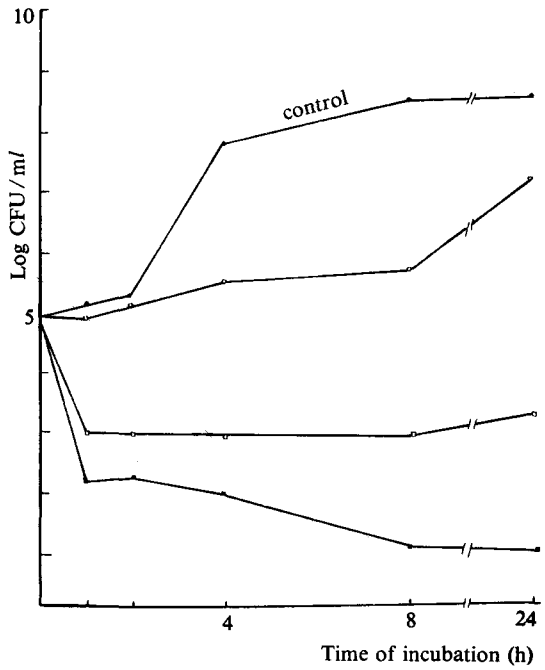

Fig. 2. Killing curves constructed from colony counts after incubation of *Pseudomonas aeruginosa* strain No. 2 with cephradine 31.25 μg/ml (○), netilmicin 8.0 μg/ml (□) and cephradine-netilmicin 7.81 + 2.0 μg/ml (■) combination.

flask with combination showed much less regrowth than that containing netilmicin alone (Fig. 3).

Emergence of resistance

The three strains of *Pseudomonas aeruginosa* maintained their sensitivity to the two antibiotics, without any variation, throughout all killing curve experiments.

Effect of cephradine (75 mg/kg), netilmicin (50 mg/kg) and their combination on serum urea and serum creatinine level of rabbits

Table III showed that cephradine alone and netilmicin alone led to a significant increase in the levels of both urea and creatinine in rabbit serum compared with the control, indicating a nephrotoxic effect. Meanwhile, the combined effect of both antibiotics on serum urea and serum creatinine levels was not significantly different from that of either cephradine alone or netilmicin alone but still significantly higher than that of the control.

DISCUSSION

The most serious problem which objects the use

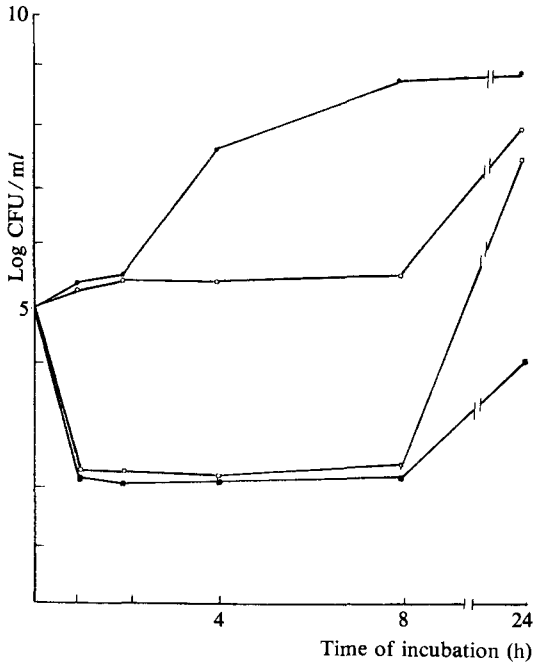

Fig. 3. Killing curves constructed from colony counts after incubation of *Pseudomonas aeruginosa* strain No. 3 with cephradine 62.5 μg/ml (○), nitelmicin 16.0 μg/ml (□) and cephradine-netilmicin 15.62 + 4.0 μg/ml (■) combination.

Table III. Effects of intraperitoneal administration of cephradine (75 mg/kg), netilmicin (50 mg/kg) alone and in combination on rabbit serum urea and creatinine levels

Treatment	Serum level of urea (mg/dl)	Creatinine (mg/dl)
Control group	42.6 ± 2.28	0.98 ± 0.082
Cephradine	67.2 ± 3.23*	1.65 ± 0.140*
Netilmicin	69.3 ± 4.20*	1.59 ± 0.130*
Combination	64.5 ± 3.80*	1.62 ± 0.15

Levels were expressed as mean values of six rabbits ± S.E.
*Significant difference from the control at $p < 0.05$.

of cephalosporin-aminoglycoside combination is the synergistic nephrotoxicity[4]. On the other hand, the use of such combination is very important in many cases where the infections are caused by organisms resistant to commonly used single antibiotics specially in complicated urinary tract infections.[13] The results obtained from the present study are in general agreement with those of other investigators who found that cephalosporin-aminoglycoside combination has a synergistic antipseudomonal activity in 30% of the isolates tested[14] or 62-69% of the isolates tested[15] or 100% of the used isolates[6] and this latter percent (100%) is in complete agreement with that of our results. However, our results indicated that beside the synergistic activity of netilmicin-cephradine combination against the three isolates, both serum creatinine and urea levels in the rabbits receiving this combination were not significantly different from those of the rabbits received either cephradine alone or netilmicin alone. The nephrotoxicity of both aminoglycosides and cephalosporins was reported by many authors. Smith *et al.*[17] stated that approximately 8-26% of the patients who received an aminoglycoside for more than several days would develop renal impairment which was always reversible. At the same time, Barza[18] reported that cephalosporins are potentially nephrotoxic agents although they are not nearly as toxic to the kidney as are the aminoglycosides. The different nephrotoxicity of cephradine-netilmicin combination may suggest that synergistic nephrotoxicity of cephalosporin-aminoglycoside combination is not a simple addition.

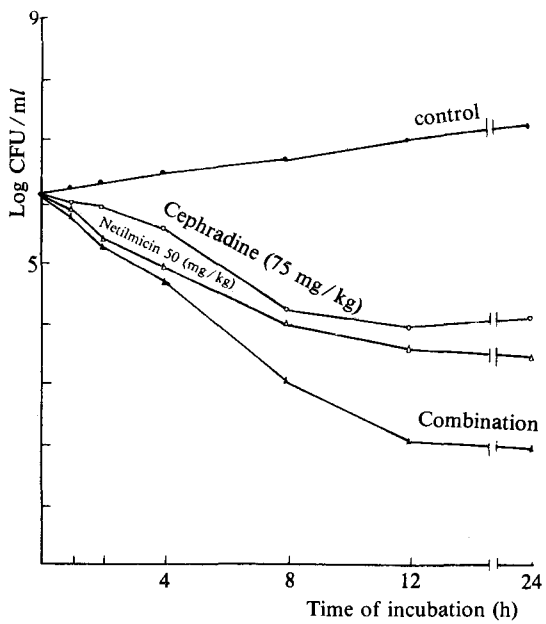

Fig. 4. Bactericidal activity of the sera of the rabbits receiving cephradine, and netilmicin alone and in combination against *Pseudomonas aeruginosa*.

LITERATURE CITED

1. Jawetz, E.: Combined antibiotic action: some definitions and correlations between labora-

tory and clinical results. *Antimicrob. Agents Chemother.* **7,** 302 (1967).
2. Moellering, R.C.: Antimicrobial synergism-an elusive concept. *J. Infect. Diseases* **140,** 639 (1979).
3. Hallander, H.O., Dornbusch, K., Gezelius, L., Jacobson, K. and Karlsson, I.: Synergism between aminoglycosides and cephalosporins with antipseudomonal activity: interaction index and killing method. *Antimicrob. Agents Chemother.* **22,** 743 (1982).
4. Wade, J.C., Simth, C.R., Petty, B.G., Lipsky, J.J.: Cephalothin plus an aminoglycoside is more nephrotoxic than methicillin plus an aminoglycoside. *Lancet.* **2,** 604 (1978).
5. Perea, E.J., Torres, M.A. and Borobio, M.D.: Synergism of fosfomycin-ampicillin aganist *Salmonella and Shigella. Antimicrob. Agents Chemother.* **13,** 705 (1978).
6. Elion, G.B., Singer, S. and Hitchings, G.H.: Antagonists of nucleic acid drevatives VIII synergism in combinations of biochemically related antimetabolites. *J. Biolog. Chem.* **308,** 477 (1954).
7. Phillips, I. and Warren, C.: Activity of sulfamethoxazol and trimethoprim against *Bacteroides fragillis. Antimicrob. Agents Chemother.* **9,** 736 (1967).
8. Rosenblatt, J.E. and Stewart, R.R.: Combined activity of sulfamethoxazol, trimethoprim and polymyxin B against gram-negative bacilli. *Antimicrob. Agents Chemother.* **6,** 84 (1974).
9. Libke, R.D., Regamy, C., Clarke, J.T. and Kirby, W.M.M.: Synergism of carbenicillin and gentamicin aganist enterococci. *Antimicrob. Agents Chemother.* **4,** 564 (1973).
10. Van Lathem, J., Lagast, H. and Klastersky, J.: Serum bactericidal activity of ceftazidin and cefoperazone alone and in combination with amikacin aganist *Pseudomanas aerugiosa* and *Kelbsiella pneumoniae. Antimicrob. Agents Chemother.* **23,** 435 (1983).
11. Patton, C.J. and Crouch, S.R.: Spectrophotometric and kinetics investigation of the Berthelot reaction for the determination of ammonia. *Anal. Chem.* **49,** 464 (1977).
12. Husdan, H. and Rapoport, A.: Estimation of creatinine by the Jaffe reaction. A comparison of three methods. *Clin. Chem.* **14,** 222 (1968).
13. Penn, R.G., Preheim, L.C., Sanders, C.C. and Giger, D.K.: Comparison of moxalactam and gentamicin in the treatment of complicated urinary tract infections. *Antimicrob. Agents Chemother.* **24,** 494 (1983).
14. Neu, H.C., Fu, K.P., Aswapokee, N. and Kung, K.: Comparative activity and β-lactamase stability of cefoperazone, a piperazine cephalosporine. *Antimicrob. Agents Chemother.* **16,** 150 (1979).
15. Pavillard, E.R. and Miles, H.: A study of cefoperazone alone and in combination with tobramycin aganist *Pseudomonas aeruginosa. Clin. Therapeut.* **3,** 134 (1980).
16. Hoogkamp-Korstanje, J.A.A., Pot, C.M. and Westerdaal, A.A.: *In vitro* activity of cefoperazone and penicillin alone and in combination with aminoglycosides aganist *Pseudomonas aeruginosa. J. Antimicrob. Chemother.* **8,** 101 (1981).
17. Simth, C.R., Lipsky, J.J., Laskin, O.L., Hellman, D.B., Mellits, E.D., Longstreth, J. and Leitman, P.S.: Double-blind comparison of the nephrotoxicity and auditory toxicity of gentamicin and tobramycin. *New Eng. J. Med.* **302,** 1106 (1980).
18. Barza, M.: The nephrotoxicity of cephalosporins: an overview. *J. Infect. Diseases* **143,** 1868 (1978).