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Rapid Communication

The effects of tobramycin, amiloride, sodium cholate and sodium deoxycholate on the growth inhibitory activity of ciprofloxacin against a respiratory tract isolate of *Pseudomonas aeruginosa*

David S. Jones [1] and Karen N. Shaffer

School of Pharmacy, University of Otago, P.O. Box 913, Dunedin (New Zealand)

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Summary

The effects of amiloride, tobramycin, sodium deoxycholate and sodium cholate on the growth inhibitory activity of ciprofloxacin against a respiratory tract isolate of *Pseudomonas aeruginosa* were examined in vitro. All agents at subinhibitory concentrations significantly enhanced the growth inhibitory activity of ciprofloxacin (0.0625 μg/ml, 1/4 MIC) for 12 h.

Cystic fibrosis (CF) is reported to be the most common lethal genetic disorder amongst white Caucasians and is characterised by chronic pulmonary disease, pancreatic insufficiency and elevated sweat electrolytes (Kelly and Lovato, 1984). Colonisation of the respiratory tract of CF patients with *Pseudomonas aeruginosa* occurs during infancy or early life and it is now accepted that this organism contributes to the lung damage seen in CF patients (Friend, 1986). Consequently, antimicrobial chemotherapy is frequently targeted against this organism and includes the use of β-lactams, e.g., ceftazidime, carbenicillin, piperacillin, and/or aminoglycosides, e.g., gentamicin, tobramycin (Kelly and Lovato, 1984). However, one drawback of these antimicrobial agents is the requirement of parenteral administration. Ciprofloxacin is a member of the new generation of 4-quinolones which possesses a broad spectrum of antibacterial activity (including *Ps. aeruginosa*) and, after oral administration, exhibits wide-spread distribution to most tissues and body fluids (Jones, 1988). The possible use of ciprofloxacin in acute exacerbations of pulmonary disease has been clinically investigated (Rubio and Shapiro, 1986; Hodson et al., 1987) and clinical improvements, comparable to conventional therapy with an anti-pseudomonal penicillin plus an aminoglycoside, were observed in the majority of patients (> 80%). However, due to the persistence of ciprofloxacin in the sputum of patients, many *Ps. aeruginosa* isolates developed resistance thus leading to clinical treatment failures (Scully et al., 1987). The mechanisms of resistance of *Ps. aeruginosa* to ciprofloxacin are currently the sub-

Correspondence to: D.S. Jones, 8, Hollybrook Park, Glengormley, Newtownabbey, Northern Ireland, U.K.
[1] *Present address:* Norbrook Laboratories Ltd., Station Works, Camlough Road, Newry, Northern Ireland, U.K.

ject of several investigations and are thought to involve mutations affecting DNA gyrase (the primary site of antibacterial action), mutations affecting OMPF production and/or permeability and mutations affecting accumulation of ciprofloxacin within the microbial cell (Celesk and Robillard, 1989; Piddock and Wise, 1989). Consequently, there may be a clinical role for agents which enhance the activity of ciprofloxacin against *Ps. aeruginosa*.

Therefore, the aim of this study was to investigate whether tobramycin, amiloride (two cationic agents), sodium deoxycholate and sodium cholate (two anionic surfactants) may enhance the growth inhibitory effects of ciprofloxacin against a clinical isolate of *Ps. aeruginosa* from a diagnosed respiratory tract infection of a cystic fibrosis patient. The selection of these agents followed preliminary investigations to identify potential agents that enhance the growth inhibitory activity of ciprofloxacin against *Ps. aeruginosa* in vitro. The agents initially screened for such activity included tobramycin, amiloride, sodium, deoxycholate, sodium cholate, propylene glycol, lysolecithin, lecithin, dipalmitoylphosphatidylcholine, glycine, cetrimide, sodium lauryl sulphate and polysorbate 80. Tobramycin, amiloride, sodium cholate and sodium deoxycholate were finally selected because of their optimal ability to enhance the growth activity of ciprofloxacin and their relatively low toxicity.

Ciprofloxacin was donated by Bayer (Australia) Ltd. Tobramycin (Nebcin^{TM}) was a gift from Eli Lilly (New Zealand) Ltd. All other chemicals were obtained from Sigma Chemical Co., St. Louis, U.S.A. and were AnalaR or equivalent quality. All solutions were prepared for use on the same day by dissolving the appropriate weight of powder in sterile deionised water.

An isolate of *Ps. aeruginosa* from a clinically diagnosed respiratory tract infection of a cystic fibrosis (CF) patient was employed in this study. Storage was performed on the surface of glass beads at −80°C and when required, one glass bead was transferred into prewarmed Mueller-Hinton broth (Difco) and incubated at 37°C in an orbital incubator (150 oscillations/min) for 18 h. The culture was then centrifuged (2000 × *g*, 15 min), washed once with and resuspended in sterile water to a cell density of approx. 1×10^7 cfu/ml.

The minimum inhibitory concentrations (MIC) of ciprofloxacin, tobramycin, amiloride, sodium cholate and sodium deoxycholate were performed using a macrodilution method as described by Jones et al. (1985). Stationary phase *Ps. aeruginosa* (approx. 3×10^8 cfu/ml) were inoculated into Mueller-Hinton broth containing doubling dilutions of the specified agents described above to produce a final viable organism count of approx. 1×10^6 cfu/ml. In addition, a positive control was prepared, i.e., broth inoculated with *Ps. aeruginosa*. The tubes were then incubated at 37°C for 24 h and the MIC accepted as the lowest concentration of agent which completely inhibited growth.

To examine the effect of amiloride, tobramycin, sodium deoxycholate and sodium cholate on the growth inhibitory activity of ciprofloxacin a turbidometric method was used. Into 16 ml Mueller-Hinton broth was added *Ps. aeruginosa* (2 ml, approx. 1×10^7 cfu/ml) and 2 ml of solution containing sterile water or the appropriate concentrations of ciprofloxacin in the presence or absence of either amiloride (25, 50 μg/ml), tobramycin (0.1 μg/ml), sodium cholate (215, 431 μg/ml) or sodium deoxycholate (207, 415, 829, 1660 μg/ml). Mueller-Hinton broth was also prepared containing these concentrations of amiloride, tobramycin, sodium deoxycholate and sodium cholate, however, in the absence of ciprofloxacin. All media were then incubated at 37°C and, at selected time intervals, samples were removed and the absorbance determined at 650 nm, using Mueller-Hinton broth as the blanking solution.

In the statistical analysis of the results, the absorbances of cultures of *Ps. aeruginosa* grown in Mueller-Hinton broth alone were assumed to represent 100% growth at each time interval. Consequently, the growth inhibitory effects of ciprofloxacin alone, and in the presence of cationic or anionic agents are expressed as percentages of this value. The effect of the presence of either tobramycin, amiloride, sodium cholate or sodium deoxycholate on the growth inhibitory

concentration of ciprofloxacin may be assessed using a Chi-squared analysis, comparing the percentage inhibition of *Ps. aeruginosa* grown in the presence, to that in the absence, of these agents. Values of $p < 0.05$ were assumed to be significant.

The minimum inhibitory concentration of ciprofloxacin against the isolate of *Ps. aeruginosa* used in this study was 0.25 μg/ml. The minimum inhibitory concentrations of tobramycin, amiloride, sodium deoxycholate and sodium cholate were 1.0, > 80, > 6500.0 and > 1700.0 μg/ml, respectively. In this present study, ciprofloxacin (0.0625 μg/ml, 1/4 MIC) inhibited the growth of this organism (i.e., reduced the growth) by 75.0–83.3% after 6 h and 38.1–69.1% after 12 h, whereas, at a lower concentration (0.03125 μg/ml, 1/8 MIC) the percentage growth inhibitions at 6 and 12 h were 42.9 and 18.2, respectively. Both cationic agents, tobramycin (0.1 μg/ml) and amiloride (50 μg/ml) and anionic agents, sodium deoxycholate (207, 415, 829, 1660 μg/ml) and sodium cholate (215, 431 μg/ml) significantly enhanced the growth inhibitory activity of ciprofloxacin (0.0625 μg/ml) against *Ps. aeruginosa* for the duration of this study, i.e., 12 h. In addition, amiloride (25 μg/ml) significantly enhanced the inhibitory activity of ciprofloxacin (0.03125 and 0.0625 μg/ml) for 6 h. Amiloride, sodium deoxycholate and tobramycin exhibited limited inhibitory effects on growth, however, with the exception of tobramycin (0.1 μg/ml) after 6 h, these were not significantly different from cells grown in the absence of these agents, i.e., in Mueller-Hinton broth containing sterile water alone.

The abilities of tobramycin and amiloride to enhance the growth activity of ciprofloxacin against *Ps. aeruginosa* are interesting given that all three agents are frequently prescribed to cystic fibrosis patients. Tobramycin has been previously reported to enhance the bactericidal activity of ciprofloxacin against *E. coli* (Lewin and Smith, 1989) which these authors suggested to be due to an increased quinolone uptake caused by aminoglycoside-induced outer membrane permeability changes. Indeed, aminoglycosides have been reported to enhance the outer membrane permeability of *Ps. aeruginosa* (Hancock and Wong, 1984). Therefore, the findings of this present study are of no suprise and further illustrate the synergy between these two compounds.

Nebulised amiloride has been reported to produce a clinical improvement in cystic fibrosis, i.e., a slowing of the loss of forced vital capacity and, improvements in sputum viscosity and elasticity (Knowles et al., 1990) and therefore its use in this disease may be beneficial. The effective concentration of amiloride reported in CF patients after

TABLE 1

The effects of amiloride (A), tobramycin (Tob), sodium cholate (NaC) and sodium deoxycholate (NaD) on the growth inhibitory activity of ciprofloxacin against Pseudomonas aeruginosa (isolated from a diagnosed respiratory tract infection from a cystic fibrosis patient)

Ciprofloxacin concentration (μg/ml)	Concentration of additive	% growth inhibition [a] after incubation at 37°C for	
		6 h	12 h
0 (water)	0 (water)	0	0
0.0625	0	75.0	61.9
0.0625	NaC 215 μg/ml	87.5	71.4
0.0625	NaC 431 μg/ml	95.0	76.2
0	NaC 431 μg/ml	0	0
0 (water)	0	0	0
0.0625	0	75.9	69.1
0.0625	NaD 207 μg/ml	100.0	96.7
0.0625	NaD 415 μg/ml	100.0	100.0
0.0625	NaD 829 μg/ml	100.0	100.0
0.0625	NaD 1660 μg/ml	100.0	100.0
0	NaD 1660 μg/ml	0#	9.5
0 (water)	0	0	0
0.0625	0	83.3	38.1
0.0625	A 25 μg/ml	100.0	38.1
0.0625	A 50 μg/ml	100.0	100.0
0	A 25 μg/ml	0	2.4
0	A 50 μg/ml	12.0	0
0.03125	0	42.9	18.2
0.03125	A 25 μg/ml	100	10.0
0 (water)	0	0	0
0.0625	0	80.0	64.3
0.0625	T 0.1 μg/ml	96.7	35.7
0	T 0.1 μg/ml	33.0	9.5

[a] Absorbances of *Pseudomonas aeruginosa* cultures grown for 6 and 12 h in the absence of antimicrobial agents accepted to be 100%; see text.

nebulisation of a 5×10^{-3} M solution was 1×10^{-4} M, i.e., 28.4 μg/ml (Waltner et al., 1987). Therefore, as enhancement of activity of ciprofloxacin was observed in this study in the presence of 25–50 mg/ml amiloride, co-administration of these agents may be clinically useful in the treatment of infection and additionally for the simultaneous improvement of respiratory function in cystic fibrosis.

Sodium deoxycholate and sodium cholate are anionic surfactants which are constituents of bile (Attwood and Florence, 1983). Amongst their physical properties, anionic surfactants accumulate at the interface between two phases, e.g., aqueous phase and microbial outer surface. In addition, these agents are known to alter the permeability or integrity of biological membranes (Attwood and Florence, 1983). Therefore, this interaction with membranes may account for the ability of these surfactants (and others, e.g., sodium lauryl sulphate and cetrimide, results not shown) to enhance the activity of ciprofloxacin. On a molar basis, a greater enhancement of inhibitory activity was observed with sodium deoxycholate than sodium cholate. This may reflect the relative toxicities of the two compounds as trihydro bile salts have been reported to be more toxic to biological membranes than their dihydro derivatives (Attwood and Florence, 1983). The effects of sodium cholate and sodium deoxycholate on the outer membrane permeability of *Ps. aeruginosa* are under current investigation.

Therefore, this study has shown that the growth inhibitory activity of ciprofloxacin against a clinical isolate of *Ps. aeruginosa* may be enhanced by the presence of the cationic agents, tobramycin and amiloride, and the anionic agents, sodium deoxycholate and sodium cholate. Consequently, inhibition of the growth of this microorganism (in the presence of these agents) may occur using lower concentrations of ciprofloxacin. Given the relatively low toxicity of these agents, these findings may be of therapeutic significance in the treatment of *Ps. aeruginosa* infections of the respiratory tract of, e.g., cystic fibrosis patients. Further investigations are ongoing to examine the efficacies of these combinations against a wider range of isolates of *Ps. aeruginosa* from a variety of clinical conditions, e.g., wounds, urinary tract and ocular infections and, in addition, to examine the effects of such drug combinations on microbial viability.

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