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Short communication

Interaction between 3,5-diacetyl-1,4-dihydropyridines and ampicillin, and erythromycin on different *E. coli* strains

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Abstract

Eleven analogues of nifedipine (NP) showed synergistic interactions with ampicillin (Ap) and erythromycin (Er) on *Escherichia coli* K12LE140/F'lac. The antibacterial effect of Ap was enhanced by most analogues but compound (G9) and (±)-verapamil (VP) were antagonistic. Two of the 11 compounds (G7, G8) were synergistic with Er and four were additive. With a sensitive clinical isolate of *E. coli* Gy-1/$Ap_{sens}Er_{res}$, compound G1 antagonized the antibacterial effect of Ap and a synergistic effect was found in the combination of Er with G4, G5, G6 or G7. None of the drugs had any effect on a multidrug resistant (MDR) clinical isolate of *E. coli* Gy-2/$Ap_{res}Er_{res}$.

Keywords: Synergism; Acetyldihydropyridines; Antibiotics; Resistance modifiers; *E. coli*

1. Introduction

Various studies have been made to increase the efficiency of antimicrobial agents. A combination of methdiazine (Md) a phenothiazine derivative and streptomycin (Sm), ampicillin (Ap), erythromycin (Er) or tetracycline (Tc) was synergistic against *Vibrio cholerae* 14033 [1]. Similarly, significant synergism was found between promazine (Pr), a phenothiazine derivative possessing antimicrobial activity, and Sm, Ap and Er or Tc against *Salmonella typhimurium* NCTC 74 infection in mice [2].

In a previous study, we found that (±)-verapamil (VP) plus promethazine were effective resistance modifiers on a laboratory strain of *Escherichia coli* by plasmid elimination [3].

The resistance modifier effect of VP is seen in bacteria [4], fungi [5,6], protozoa [7] and cancer cells [8] but adverse effects occurred. To improve specificity, new nifedipine (NP) analogues were synthesized on the basis of previous results [9] and tested on a laboratory strain of *E. coli* and two clinical isolates. The interaction between antibiotics and newly synthetized NP analogues were studied and evaluated by the chequerboard test.

2. Materials and methods

2.1. Chemicals

Eleven acetyldihydropyridines AcDHP (G1–11) of NP analogue were synthesized as previously described [9] (Fig. 1).

2.2. Antibiotics and resistance modifiers

The following antibiotics were obtained from the companies indicated: Ap (Beechaem Research Laboratories, England); Er (Richter Gedeon RT, Budapest, Hungary).

The resistance modifiers were obtained from the companies indicated; (±)-VP (Chinoin, Budapest, Hun-

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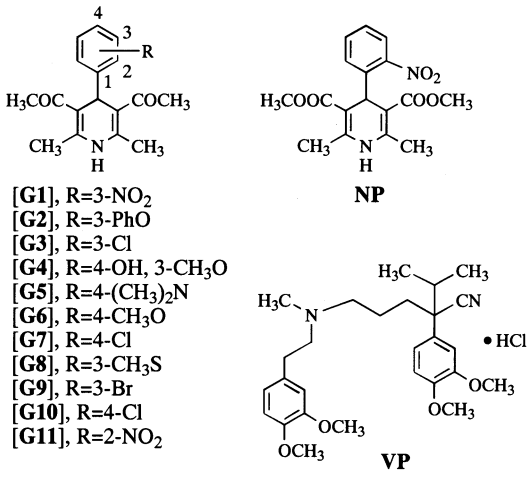

Fig. 1. Structures of 3,5-diacetyl-1,4-dihydropyridines (G1–11), (±)-VP and NP.

gary); NP (Aldrich Chemical Co., Inc., Milwaukee, WI, USA).

2.3. Bacterial strains

A laboratory strain of *E. coli* K12LE140/F'lac and two clinical isolates (*E. coli* Gy-1/$Ap_{sen}Er_{res}$ and *E. coli* Gy-2/$Ap_{res}Er_{res}$) were kindly provided by the public health institute of Csongrad county.

2.4. Measurement of antibacterial activity

The antibacterial effect of the tested compounds was studied in modified minimal tryptone yeast extract (MTY) liquid media [4], containing 1.0 g NH_4Cl, 7.0 g K_2HPO_4, 3.0 g NaH_2PO_4, 0.8 g NaCl, 1.0 g D-glucose, 10.0 g Bacto trytone (Difco) and 1.0 g yeast extract (Difco) in 1.0 l distilled water at pH 7.2.

An overnight preculture of the laboratory strain and two clinical isolates of *E. coli* were diluted 10^{-4}-fold and 0.1 ml (ca. 5×10^3 bacteria) was inoculated into 5.0 ml of MTY broth containing various concentrations of the different compounds. The cultures were grown at 37 °C without shaking. Minimum inhibitory concentrations (MIC) were read after 24 h incubation.

Three bacterial strains were tested by the microdilution chequerboard technique described by Eliopoulus and moellering [10]. Briefly, bacterial dilutions from the logarithmic-growth phase were prepared and subsequently distributed into micrometer trays containing various drug regimen concentrations. The final inoculum size in the micrometer trays was approximately 10^5 colony forming unit (CFU)/ml.

Inoculated micrometer trays were incubated at 37 °C for a period of 24 h and were then read for inhibition of bacterial growth. In order to evaluate the outcome of the drug combination, fractional inhibitory concentration (FIC) indices were calculated as $FIC_A + FIC_B$, when FIC_A and FIC_B represent the minimum concentrations that inhibited inoculum growth for drugs A and B, respectively [9]. Individual chequerboard runs were replicates, a mean FIC index was calculated and applied to a commonly utilized definition of synergy, and classified as either synergistic (≤ 0.5), additive (0.51–1.0), indifferent (1.01 but ≤ 4.0), or antagonistic (above 4.0) (Table 1).

Table 1
MICs by synergistic effect of 3,5-acetyl-1,4-dihydropyridines 5 μg/ml and ampicillin (Ap) or 8 μg/ml erythromycin (Er) sensitive and resistant *E. coli* strains

	E. coli					
	K12LE140/F'lac		Gy-1/$Ap_{sens}Er_{res}$		Gy-2/$Ap_{sens}Er_{res}$	
	AMP	ERY	AMP	ERY	Ap	Er
Antibiotic alone	4	8	8	64	256	> 64
+G1	1	8	64	32	> 64	> 64
+G2	2	4	8	32	> 64	> 64
+G3	1	4	8	32	> 64	> 64
+G4	1	8	8	16	> 64	> 64
+G5	2	8	8	16	> 64	> 64
+G6	2	8	8	16	> 64	> 64
+G7	1	2	8	16	> 64	> 64
+G8	1	2	8	32	> 64	> 64
+G9	> 8	8	8	32	> 64	> 64
+G10	1	4	8	32	> 64	> 64
+G11	1	4	8	32	> 64	> 64
+(±)-VP	32	4	4	32	> 64	> 64
+NP	2	4	8	32	> 64	> 64

3. Results and discussion

3.1. Antibacterial activity of Ap, AcDHP (G1–11), VP, NP and Er

MIC values of AcDHP after 24 h were measured on three different *E. coli* strains and the results are shown in Table 1. No antibacterial effect was seen by the non-antibiotics up to a concentration of 100 μg/ml. Table 1 shows the results of the chequerboard studies.

3.2. Combination effect of AcDHP (G1–11) with Ap

A synergistic effect of AcDHP was seen with Ap against *E. coli* K12LE140/F'lac after 24 h, when combination with seven AcDHP reduced the Ap MIC to 1 mg/l and the MIC of NP to 2 mg/l. Two compounds G9 (MIC > 8 mg/l) and VP (MIC: 32 mg/l) were less effective than the others. The most effective compounds were as follows: G1, G3, G4, G7, G8, G10 and G11.

The MICs of Ap against a clinical isolate (*E. coli* Gy-1/$Ap_{sens}Er_{res}$) after 24 h with combination of G1 were antagonistic (MIC for Ap 64 mg/l) (Table 1). Ten other AcDHP (G1–11) (MIC: 8 mg/l) and NP (MIC: 8 mg/l) were not synergistic and only VP (MIC: 4 mg/l) had an additive effect.

3.3. Combination effect of AcDHP (G1–11) with Er

AcDHP (G1–11) had a synergistic effect with Er against *E. coli* K12LE140/F'lac after 24 h, the MICs of G7 (MIC: 2 mg/l) and G8 (MIC: 2 mg/l) were synergistic whereas the MIC values of G2, G3, G10, G11, VP and NP were additive (4 mg/l). The compounds G1, G4, G5, G6 and G9 were ineffective in combination with Er against *E. coli* K12LE140/F'lac strain (Table 1). Compound G7 was the most effective of the eleven AcDHP (G1–11), VP and NP with Ap or Er against *E. coli* K12LE140/F'lac after 48 h (Table 1).

Combination of Er with eleven AcDHP (G1–11) (MIC: 16–32 mg/l), VP (MIC: 32 mg/l) and NP (MIC: 32 mg/l) had no effect on the MIC of Er (Table 1).

The combinations of AcDHP (G1–11) with the two antibiotics against clinical isolate *E. coli* Gy-2/$Ap_{res}Er_{res}$ showed no differences (Table 1).

4. Conclusions

Synergistic or additive effects of AcDHP (G1–11) with Ap or Er have been shown on *E. coli* K12LE140/F'lac and *E. coli* Gy-1/$Ap_{sens}Er_{res}$ but, not on *E. coli* Gy-2/$Ap_{res}Er_{res}$. These effects of synergistic or additive combinations are supported by additional experiments, in which trimeprazine exhibited significant synergistic antimicrobial activity when combined with either trimethoprim or sulfathiazole [11]; development of cross-resistance by administration of non antibiotics with antibiotics has also been repeated [12]. The present paper clearly defines the effects of combinations of AcDHP (G1–11) analogues with Ap or Er, which are of some interest.

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