

IN VITRO SYNERGY
OF ACTITHIAZIC ACID,
AN ANTIMETABOLITE OF BIOTIN,
COMBINED WITH CEPHALEXIN

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(Received for publication May 20, 1983)

In our screening for cell-wall active antibiotics we came across a *Streptomyces* species HPL No. Y-28,209 whose culture filtrate showed poor antibacterial activity when tested against *Escherichia coli* 9632 in the nutrient medium but very much enhanced activity when tested in the presence of cephalixin. The antibiotic isolated from the *Streptomyces* culture filtrate was found to be actithiazic acid. In this study, evidence is presented to show that synergism exists between cephalixin and actithiazic acid against *E. coli* 9632 tested in nutrient medium.

To check synergism, tests were performed according to the checker-board pattern in liquid medium. *E. coli* 9632 was grown overnight in nutrient broth consisting of beef extract (Oxoid) 0.5%, Peptone (Sheffield) 0.5%, glucose 0.25%, NaCl 0.5%, pH 7 and diluted with fresh broth to give an optical density of 0.1 at 600 nm. 0.1 ml of this inoculum containing 10^8 cfu was added to

3.9 ml of nutrient broth containing two-fold dilutions of one drug in combination with two fold dilutions of the other. The mixture was incubated at 37°C for 18 hours and then MIC values measured. MIC values for each agent alone were also determined separately. The isobologram of the combination of actithiazic acid and cephalixin tested by checker-board method is shown in Fig. 1. The FIC index¹⁾ was around 0.3 indicating moderate synergy²⁾.

Killing curve studies on *E. coli* 9632 were carried out in nutrient broth at 37°C on a reciprocating shaker. Cephalixin and actithiazic acid were added at different stages of the growth cycle and the bacterial growth monitored by optical density measurements at 600 nm. Figs. 2 and 3 show that *E. coli* 9632 can grow in the presence of either compound alone at the concentrations tested or when actithiazic acid in the combination is not present at the initiation of the growth. When actithiazic acid was added after 1 hour of bacterial growth, no synergism with cephalixin was observed. Cephalixin did not show such preferences.

Actithiazic acid is known to interfere with biotin biosynthesis^{3,4)}. Recently, EISENBERG and HSIUNG⁵⁾ have shown that actithiazic acid inhibits the enzymatic conversion of dethiobiotin to biotin in resting cell preparations. Although we do not have direct evidence to prove the role of actithiazic acid in the actithiazic acid-cephalixin synergy, it is speculated that *E. coli* cells growing in the presence of actithiazic acid are deprived of biotin and grow as defective cells and thus become susceptible to much lower concentrations of cephalixin compared to the *E. coli* cells

Fig. 1. Isobologram showing synergy of actithiazic acid and cephalixin against *E. coli* 9632. Figures given in brackets are FIC index values.

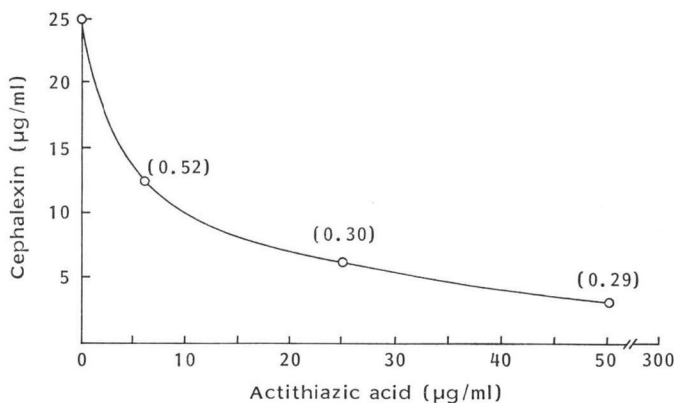
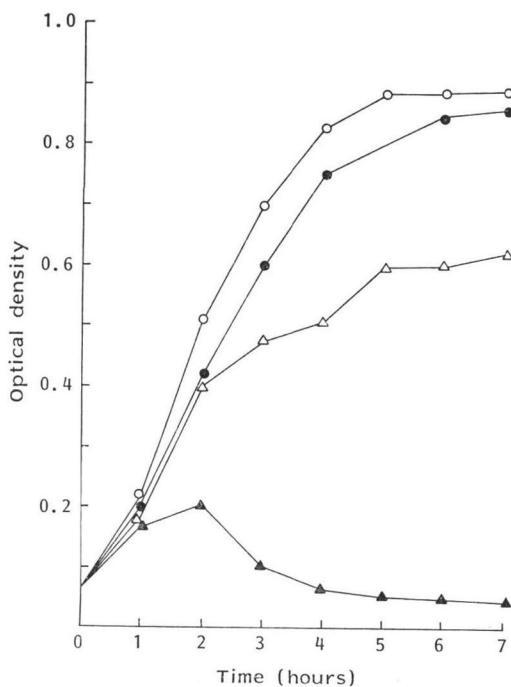


Fig. 2. Killing curves showing synergy of actithiazic acid and cephalixin against *E. coli* 9632.

All additions were done at zero hour. No addition (○); cephalixin 5 μg/ml (●); actithiazic acid 10 μg/ml (△); cephalixin 5 μg/ml and actithiazic acid 10 μg/ml (▲).



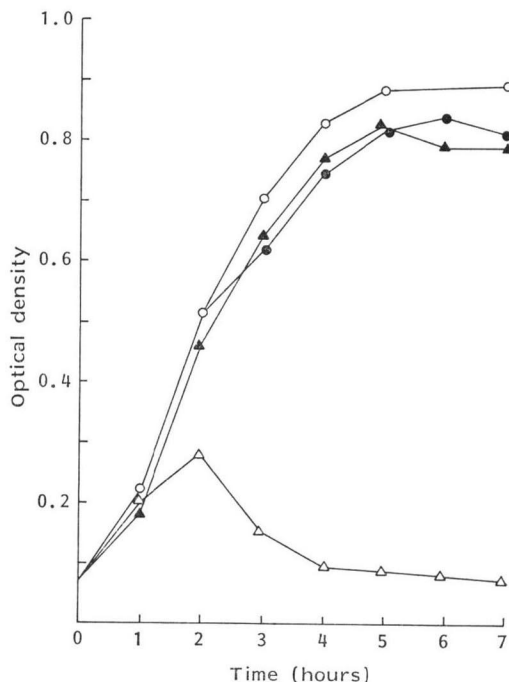
growing normally in absence of actithiazic acid. This speculation is based on our observation that the synergistic activity is reversed by biotin and that actithiazic acid fails to show synergism when it is added after the cells have grown for 1 hour. Probably in the latter case, a sufficient pool of biotin is synthesized during this period to allow further growth to take place normally. The addition of actithiazic acid at this stage therefore does not result in any biotin-depriving effect to make the cells susceptible to low concentrations of cephalixin and hence does not exhibit synergism. The damage due to biotin deprivation probably leads to an alteration in the cell wall/cell membrane integrity of *E. coli* particularly its lipid component whose synthesis is known to be affected by nonavailability of biotin⁶⁾.

References

- 1) DOUGHERTY, P. F.; D. W. YOTTER & T. R. MATTHEWS: Microdilution transfer plate technique for determining *in vitro* synergy of anti-

Fig. 3. Killing curves showing synergy of actithiazic acid and cephalixin against *E. coli* 9632.

Additions were done at the time indicated. No addition (○); cephalixin 5 μg/ml at zero hour and actithiazic acid 10 μg/ml at 1 hour (●); actithiazic acid 10 μg/ml at zero hour and cephalixin 5 μg/ml at 1 hour (△); cephalixin 5 μg/ml and actithiazic acid 10 μg/ml both at 1 hour (▲).



microbial agents. *Antimicrob. Agents Chemother.* 11: 225~228, 1977

- 2) ALLEN, J. G.; F. R. ATHERTON, M. J. HALL, C. H. HASSALL, S. W. HOLMES, R. W. LAMBERT, L. J. NISBET & P. S. RINGROSE: Phosphonopeptides as antibacterial agents, alaphosphin and related phosphonopeptides. *Antimicrob. Agents Chemother.* 15: 684~695, 1979
- 3) GRUNDY, W. E.; A. L. WHITMAN, E. G. RDZOK, E. J. RDZOK, M. E. HANES & J. C. SYLVESTER: Actithiazic acid. I. Microbiological studies. *Antibiot. Chemother.* 2: 399~408, 1952
- 4) UMEZAWA, H.; K. OIKAWA, Y. OKAMI & K. MAEDA: Thiazolidone antibiotic as an antimetabolite to biotin. *J. Bacteriol.* 66: 118~119, 1953
- 5) EISENBERG, M. A. & S. C. HSIUNG: Mode of action of the biotin antimetabolites. Actithiazic acid and alpha methyl dethiobiotin. *Antimicrob. Agents Chemother.* 21: 5~10, 1982
- 6) LYNEN, F.: The role of biotin-dependent carboxylations in biosynthetic reactions. *Biochem. J.* 102: 381~400, 1967