NOTES

LYSINE AND LYSINE ANALOG POTENTIATION OF ANTIBIOTIC AND ANTIMICROBIAL ACTIVITY

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Recently we reported that L-lysine potentiated the antibacterial activity of N^5-hydroxy-L-arginine against *Escherichia coli* (Davis 113-3), and that the inhibition by this combination could be relieved by addition of cyanocobalamin to the growth medium for this organism.[1] FISCHER *et al*[2] also noted that this combination was a potent growth inhibitor of several Gram-positive and Gramnegative bacter, and we have been interested in extending these studies to examination of lysine analogs and other antimicrobial agents.

Among the compounds used were the antibacterial[3,4] thialysine (S-(β-amino-ethyl)-L-cysteine), the antibacterial[5] DL-4-oxalysine, and β-lysine, a component of the streptothricin group of antibiotics. All of these potentiated the inhibition of growth of *E. coli* (Davis 113-3) by N^5-hydroxy-L-arginine in chemically defined medium[1] where vitamin B_{12} was the growth limiting component. Some of the data are summarized in Fig. 1 where these compounds are compared with L-lysine and hydroxylysine mentioned previously.[1] D-Lysine and ornithine were ineffective as potentiators of N^5-hydroxy-L-arginine in this test system. Similar results (Fig. 2) were obtained with *Pseudomonas aeruginosa* (ATCC 10145, a strain used for antibiotic testing) when grown in this chemically defined medium. Less elaborate studies using *Bacillus subtilis* (Marburg), *Staphylococcus aureus* (1206 and 209P), *Sarcina lutea*, *Escherichia coli* (strains B, 8 and 15[6]), *Klebsiella pneumoniae* (ATCC 10031), *Proteus mirabilis* (ATCC 12453), and *Candida albicans* (growing in the chemically defined medium supplemented with 0.25 g/liter of yeast extract) confirmed the potentiating effect of L-lysine L-thialysine and DL-oxalysine on the growth inhibitory effect of N^5-hydroxy-L-arginine.

As most Gram-positive and Gram-negative bacteria contain *meso*-diamino-pimelic acid, L-lysine or a related amino acid in their cell walls,[7] it seemed possible that a mechanism of action of thialysine might involve inhibition of incorporation of lysine into the cell wall. If this is the situation, then thialysine should act as a potentiator of those antibiotics which

Fig. 1. Potentiation by lysine and analogs of inhibition of *E. coli* (Davis 113-3) by N^5-hydroxy-L-arginine.

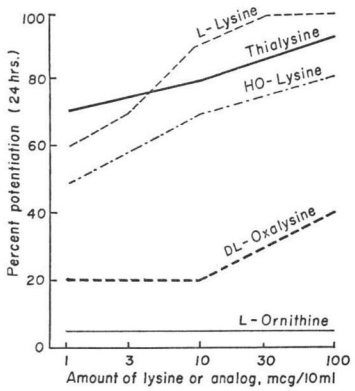

Fig. 2. Potentiation by lysine and analogs of inhibition of *Ps. aeruginosa* by N^5-hydroxy-L-arginine.

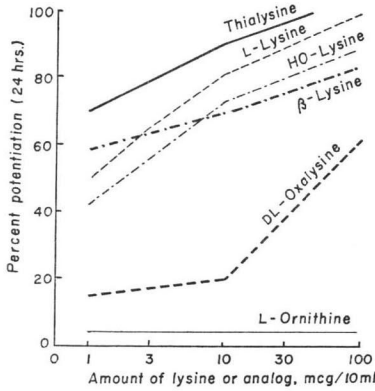

Table 1. Antibiotic potentiation by L-thialysine.
Approximate decrease in minimal inhibitory concentration (percentage)

Antibiotic	*Bacillus subtilis* (Marburg)	*Bacillus* species (Pan)	*Staphylococcus aureus* (209P)	*Sarcina lutea*	*Escherichia coli* (B)	*Pseudomonas aeruginosa* (ATCC 10145)	*Proteus mirabilis* (ATCC 12453)	*Klebsiella penumoniae* (ATCC 10031)
Penicillin G	95	95	95	95	no change	no change	no change	no change
Ampicillin	95	95	95	95	50	no change	50	75
Cephalothin	95	95	95	95	50	no change	50	50
Cycloserine	95	95	95	95	10	no change	no change	no change
Vancomycin	95	95	95	95	no change	no change	no change	no change
Lincomycin	95	95	95	95	no change	no change	no change	no change
Novobiocin	95	95	95	95	no change	no change	no change	no change
Tetracycline	95	95	95	95	no change	50	50	50
Chloramphenicol	95	95	95	95	50	no change	no change	no change
Neomycin	95	95	95	95	no change	no change	no change	no change

All evaluations done using gradient plate streak test method with salts-glucose agar supplemented with 0.25 g/liter Difco yeast extract and 0.1 mg/ml of L-thialysine.

have as their mechanism of action inhibition of cell wall biosynthesis. This was tested by the gradient plate method using a glucose-salts agar with 0.25 g/liter Difco yeast extract and 0.1 g/liter L-thialysine (this concentration did not inhibit growth of the test organisms). The test organisms were grown in nutrient broth and then the cells were washed with sterile saline solution. These washed cells were streaked on the agar surface and the change in m.i.c. determined by the differences observed with growth on gradient plates which didn't have the thialysine supplement. Some of the data collected are summarized in Table 1.

Thialysine was effective in increasing the sensitivity of these bacteria to several types of antibiotics (if the bacteria were sensitive at all), and in many instances this increase was about 20-fold, *i.e.* the m.i.c. decreased by about 95 %. When a peptone medium was used, no potentiation was noted, and when L-lysine was added to the salts-glucose basal medium at 0.1 g/liter, no potentiation was observed (an observation that was expected in view of the SHIOTA *et al*[4,8] observations). Other substances effective in reversing the L-thialysine potentiation included L-lysyl-L-phenylalanine, L-lysyl-L-valine, L-lysyl-L-lysine, and α-glycyl-L-lysine. Substances ineffective included D-lysine and L-lysyl-glycine. The potential utility of L-thialysine-antibiotic mixtures *in vivo* remains to be determined. Since L-thialysine undergoes acetylation and transamination by bacterial enzymes[9,10], it may be metabolized *in vivo* too rapidly to be an effective potentiator in infected animal systems.

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References

1) PERLMAN, D.; A.J. VLETINCK, H.W. MATHEWS & F.F. LO: Microbial production of vitamin B_{12} antimetabolites. I. N^5-Hydroxy-L-arginine from *Bacillus cereus* 439. J. Antibiotics 27: 826~832, 1974
2) FISCHER, B.; W. KELLER-SCHIERLEIN, H. KNEIFEL, W.A. KÖNIG, W. LOEFEFLER, A. MÜLLER, R. MUNTWYLER & H. ZÄHNER: Stoffwechselprodukts von Mikroorganismen. 118. δ-N-Hydroxy-L-arginin, ein Aminosäure-Antagonist aus *Nannizzia gypsea*. Arch. Mikrobiol. 91: 203~220, 1973
3) CAVALLINI, D.; C. DEMARCO, B. MONDOVI & G.F. AZZONE: A new synthetic sulfur containing amino acid: S-(aminoethyl)-cysteine. Experientia 15: 61~62, 1955
4) SHIOTA, T.; J.E. FALK & F. TIETZE: Inhibition of lysine utilization in bacteria by S (β-aminoethyl) cysteine and its reversal by

lysine peptides. Arch. Biochem. Biophys. 77: 372~377, 1958

5) McCord, T.J.; J.M. Ravel, C.G. Skinner & W. Shive: dl-4-Oxalysine, an inhibitory analog of lysine. J. Am. Chem. Soc. 79: 5693~5696, 1957

6) Schwartz, J.L. & D. Perlman: Substrate specificity of streptomycin-adenylating enzymes from gram-negative bacteria. J. Antibiotics 23: 254, 1970

7) Schleifer, K.H. & O. Kandler: Peptidoglycan types of bacterial cell walls and their taxonomic implications. Bact. Rev. 36: 407~477, 1972

8) Shiota, T.; J. Mauron, & J.E. Folk: Inhibition of lysine and lysine peptide utilization in bacteria by peptides of S-(β-aminoethyl)-cysteine. Biochim. Biophys. Acta 53: 360~365, 1961

9) Soda, K.; H. Misono & T. Yamamoto: Transamination of S-(β-aminoethyl)-l-cysteine by l-lysine: α-Ketoglutarate aminotransferase. Biochim. Biophys. Acta 177: 364~367, 1969

10) Soda, K.; H. Tanaka & T. Yamamoto: Bacterial N-acetylation of an l-lysine antagonist, S-(β-aminoethyl)-l-cysteine. Arch. Biochem. Biophys. 130: 610~616, 1969