PYRIMIDINE REVERSAL OF EMIMYCIN INHIBITION OF *ESCHERICHIA COLI*

JOHN R. DE ZEEUW and
EDWARD J. TYNAN

Medical Research Laboratories,
Chas. Pfizer & Co., Inc., Groton,
Connecticut 06340, U. S. A.

(Received for publication June 13, 1969)

During a survey seeking new antibiotics produced by Actinomycetes, an antimetabolite, active against *E. coli* and reversed principally by uracil, was isolated. The structure was assigned employing the usual methods and shown to be 2-hydroxypyrazine-4-oxide (MURAI, unpublished). The compound is therefore the previously described emimycin[1], an antibiotic first isolated in 1960 by TERAO *et al.*[2] and reported to have a broad, but weak, spectrum of activity on complex media. The circumstance that this pyrazine could antagonize metabolically essential pyrimidines in a non-fastidious organism has to our knowledge not been recognized and led us to characterize the nutritional profile of the substance in greater detail.

Materials and Methods

Emimycin was isolated in our laboratories essentially by the procedure described by TERAO *et al.*[2]. The nutritional supplements were purchased from Calbiochem.

CROOK's strain (ATCC 8739) of *E. coli* was used for the growth studies. Cell densities were determined turbidimetrically in a Klett-Summerson Colorimeter with a KF-66 filter. The defined medium contained 200 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid buffer (HEPES), 50 mM D-glucose, 20 mM $(NH_4)_2SO_4$, 200 μM $MgSO_4 \cdot 7H_2O$, 200 μM KH_2PO_4 and 5 μM each of $FeSO_4 \cdot 7H_2O$, $MnSO_4 \cdot H_2O$ and $CaCl_2 \cdot 2H_2O$. The pH was adjusted to 7.2 with KOH, supplements added, and the media sterilized by membrane filtration. Standardized inoculum was prepared from cells harvested in the middle of their exponential growth period in a nutrient broth and washed twice with the defined medium lacking glucose. For all experiments, the inoculated tubes containing 5 ml total volume were incubated at 30°C for 16 hours. Initially, the turbidity was equivalent to 0.1 Klett units. After 16 hours, a typical control tube read 110 Klett units and had a final pH of 7.0.

Results and Discussion

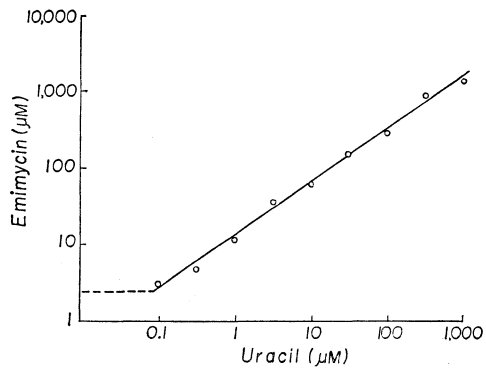
The comparative activity of emimycin and two common uracil antimetabolites is shown in Table 1. On a molar basis, emimycin is 9 times more potent than 6-azauracil but 48-fold less effective than 5-fluorouracil.

Table 1. The minimal inhibitory concentrations of various uracil antimetabolites for *E. coli* (ATCC 8739)

	MIC (mcg/ml)
5-Fluorouracil	0.03
Emimycin	1.3
6-Azauracil	12

The ability of various compounds at a concentration of 250 μM to reverse the growth inhibition by emimycin was studied. The free base, the riboside and the deoxyriboside of uracil completely reversed the inhibitory action of 250 μM emimycin. The free base, the riboside, and the deoxyriboside of cytosine as well as orotic acid partially reversed the inhibition by this level of emimycin. In contrast, thymine, thymine riboside, thyimidine, and the 20 amino acids common to protein could not reverse the

Fig. 1. Concentration of emimycin for 50 % inhibition of standard *E. coli* growth in the presence of uracil



inhibition caused by 25 μM emimycin. Adenine, guanine, hypoxanthine, xanthine, pantothenic acid, cyanocobalmin, thiamine, riboflavin, inositol, D-biotin, *p*-aminobenzoic acid, folic acid, niacinamide, and pyridoxine were also without effect.

The efficacy of uracil as a reversing agent for emimycin inhibition is shown in Fig. 1. Up to 0.1 μM, uracil supplementation caused no detectable change in the emimycin required for the effect measured (the end point of 50 % normal cell density after 16 hours of incubation). At levels of uracil greater than 0.1 μM, the interaction of emimycin and uracil is presented linearly by a log-log plot and shows the variable ratio of antimetabolite to metabolite necessary for the effect observed. The plot is simple and orderly but no mechanism of action is implied by this representation.

The data demonstrate emimycin to be a potent uracil antagonist and a research tool of considerable potential. The metabolism of this pyrazine and its confirmational homology at pertinent pyrimidine receptor sites poses an interesting study of structure-activity relationships.

Acknowledgment

We wish to thank IRENE STASKO, HENRY VAITAITIS and THEODORE NELSON for their skilled technical assistance.

References

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