INHIBITION OF IN VITRO PROTEIN SYNTHESIS BY A CARBOCYCLIC PUROMYCIN ANALOG

Robert Vince, Susan Daluge and Maxine Palm

Department of Medicinal Chemistry, College of Pharmacy
University of Minnesota, Minneapolis, Minnesota 55455

Received December 2, 1971

SUMMARY

The effect of a novel puromycin analog (I) on the poly U and poly UC directed polyphenylalanine synthesis has been examined. The inhibition of both systems by I corresponded with the inhibitory activities of puromycin. The carbocyclic analog also demonstrated inhibitory activity in three tumor systems grown in tissue culture.

Toxic manifestations, including renal lesions, have precluded the use of puromycin in the treatment of human or animal infectious diseases or neoplasms (1). The nephrotic syndrome results from small amounts of aminonucleoside produced by the hydrolytic removal of the amino acid moiety from administered puromycin (1). Recent studies demonstrate that the aminonucleoside is first monodemethylated (2) and subsequently converted to the 5'-nucleotide (3). It has been suggested that this 5'-nucleotide is responsible for the nephrotic syndrome (3).

Recently, the synthesis of the carbocyclic puromycin analog, 6-dimethylamino-9-{R-[2R-hydroxy-3R-(p-methoxyphenyl-L-alanylamino)]cyclopentyl} purine (I) was described in which the ribofuranosyl moiety of puromycin was replaced

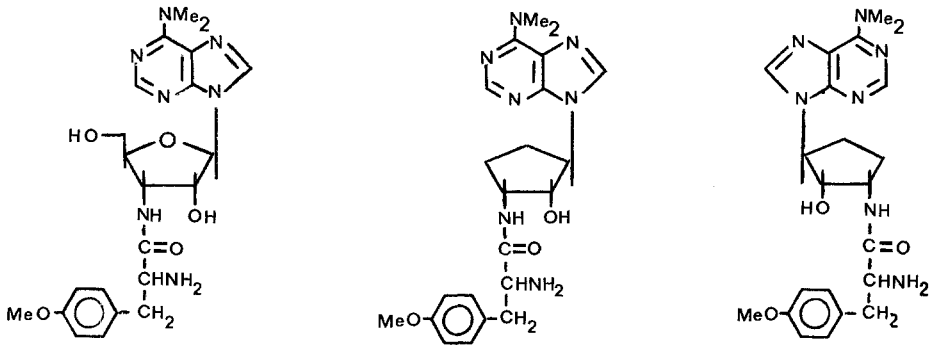

Fig. 1. Structures of puromycin, the carbocyclic analog, I, and its diasteriomer, II.

by the appropriately substituted cyclopentane ring (4). In addition, the corresponding hydroxymethyl moiety was deleted in order to render the molecule incapable of nucleotide formation. Antimicrobial testing of I demonstrated activities in the same order of magnitude as puromycin in inhibiting bacterial growth. Also, as expected, the diasterioisomer, II, did not exhibit antimicrobial activity. The aminonucleoside derived from I was completely non-toxic under conditions that cause severe nephrotoxicity with puromycin aminonucleoside (4). We now wish to report experimental evidence to support our proposal (4) that I acts as a puromycin analog by inhibiting protein biosynthesis.

MATERIALS AND METHODS

Materials. E. coli cell paste (B, late log) and s-RNA were purchased from General Biochemicals, Inc. ^{14}C-L-phenylalanine was obtained from New England Nuclear Corp. The polynucleotides were purchased from Miles Laboratories and ATP, GTP, phosphoenolpyruvate, and pyruvate kinase were purchased from Sigma. Puromycin dihydrochloride was obtained from Nutritional Biochemical Co. and chloramphenicol was generously supplied by Parke, Davis and Co.

Preparation of Ribosomes and S-100. The S-100 fraction and ribosomes were prepared by a previously reported method (5). The final ribosomal suspension was dialyzed against four changes of buffer containing 0.01M Tris-Cl, 0.01M $MgAc_2$, 0.05M NH_4Cl, 0.001M dithiothreitol (pH 7.4) - 50% glycerol for 2 days,

adjusted to 28 mg/ml and stored at -20°.

Amino Acid Incorporation. Reactions were performed in a final volume of 40 µl and contained 0.1M Tris-Cl (pH 8), 0.05M KCl, 0.01M β-mercaptoethanol, 0.005M phosphoenolpyruvate, 0.001M ATP, 0.05mM GTP, 30 µg/ml of pyruvate kinase, 2µl of S-100 fraction, 0.5 µCi/ml of ^{14}C-L-phenylalanine, 15mM $MgAc_2$, 1.2 mg/ml s-RNA 2 mg/ml of ribosomes, and 0.25 µmP/ml of poly U or 0.5 µmP/ml of poly UC (1:1). In a typical experiment a solution was made which contained all of the above components (except ribosomes and polynucleotide) and the desired concentration of inhibitor. Ribosomes (3µl) were added and the reaction was initiated by the additon of 5 µl of an appropriate polynucleotide solution. Incubations were for 30 min at 37°.

Following incubation, 30 µl was removed and placed on a 2 cm square of Whatman No. 31 ET paper. The papers were collected and immersed in 400 ml of 10% trichloroacetic acid. The papers were then heated at 90° for 15 min in 5% trichloroacetic acid. The heating was followed by two washes in 5% trichloroacetic acid and then two washes each in ethanol-ether (2:1) and ether, respectively. All washes were for 5 min (except the one at 90°) with gentle stirring while the papers were suspended in a wire basket. The papers were dried and placed in counting vials containing a toluene-based liquid scintillator (Packard Instrument Co.) and counted in a Packard Tri-Carb Model 3375 scintillation counter. Counting efficiency was approximately 86%. All counts were corrected by blanks in which either ribosomes or polynucleotide were absent. All values represent an average of triplicate determinations. The standard deviation of such replicates averaged $< \pm$ 6%.

RESULTS

The carbocyclic puromycin analog, I, inhibited the formation of polyphenylalanine in the E. coli cell free system while the diasteriomer, II, was inactive. These results are in excellent agreement with the antimicrobial studies (4). Examination of Table I reveals that the degree of inhibition exerted by I varied

with the composition of the template used. These results are consistent with the

TABLE I

INHIBITION OF L-[^{14}C]-POLYPHENYLALANINE FORMATION

Compound	Molar Concentration	% Inhibition poly UC (1:1)	% Inhibition poly U
I	10^{-6}	37	-
	10^{-5}	75	-
	10^{-4}	97	6
	10^{-3}	100	25
Puromycin	10^{-6}	39	-
	10^{-5}	78	7
	10^{-4}	99	22
	10^{-3}	100	76
Chloramphenicol	10^{-4}	79	23
	10^{-3}	94	31

relative activities of puromycin and chloramphenicol (Table I); similar observations have been reported for these antibiotics (6,7). It is interesting to note that equimolar concentrations of I and puromycin exhibit different inhibitory activities in the presence of poly U while poly UC directed polyphenylalanine is inhibited to the same extent by both compounds. No definite conclusion can be made at this time to explain these differences in relative activities.

Tissue culture studies on three tumor systems reveal that I exhibits antitumor activity while II is inactive. The concentrations (μg/ml) of I for 50% inhibition of growth against L-1210 leukemia, P-388 leukemia, and KB epidermoid carcinoma are 0.10, 0.04, and 0.27, respectively (8). In vivo antitumor testing results are not available at this time.

We conclude from these data that the hydroxymethyl moiety and the furan-

osyl ring oxygen of puromycin are not essential for activity. The removal of these moieties has led to a novel class of puromycin analogs with distinct advantages over the classical nucleoside compounds. The non-glycosidic linkage between the purine and the cyclopentane moieties render the molecule resistant to hydrolytic cleavage, and the removal of the hydroxymethyl group circumvents the problems associated with nucleotide formation.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. James W. Bodley of the Department of Biochemistry for his advice and the use of his facilities in the preparation of ribosomes for our studies. This work was supported by Grant AI08142 from the U.S. Public Health Service and Grant CA10979 from the National Cancer Institute.

REFERENCES

1. Nathans, D. (1967). "Antibiotics I, Mechanism of Action," D. Gottlieb and P. D. Shaw, Eds., Springer-Verlag New York Inc., New York, N. Y., pp 259-277.
2. Derr, R. F., Alexander, C. S., and Nagasawa, H. T. (1967). Proc. Soc. Exp. Biol. Med. 125, 248.
3. Kmetec, E. and Tirpack, A. (1970). Biochem. Pharmacol. 19, 1493.
4. Daluge, S. and Vince, R. (1972). J. Med. Chem. 15 (in press).
5. Bodley, J. W. (1969). Biochemistry 8, 465.
6. Coutsogeorgopoulos, C. (1966). Biochim. Biophys. Acta 129, 214.
7. Harris, R. J., Hanlon, J. E. and Symons, R. H. (1971). Ibid. 240, 244.
8. We wish to thank Dr. Florence White of CCNSC for the data on the L-1210 and KB systems.