INHIBITION OF NUCLEIC ACID SYNTHESIS IN L1210 CELLS BY NUCLEOSIDE ANALOGS*

Jiří BERÁNEK[a] and Edward M. ACTON[b]

[a] *Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6, and*
[b] *Stanford Research Institute International, Menlo Park, California 94025, USA*

Received March 26th, 1984

Series of pyrimidine nucleoside analogs were tested for inhibition of DNA and/or RNA synthesis at L1210 cells. The structure–activity relationship was studied at the analogs of cancerostatic agents 5-fluorouracil and arabinosylcytosine. Out of them the 5'-chloro derivatives give some promise. The inhibitory activity of cyclocytidine *vs* DNA and RNA synthesis approaches the activity of cancerostatic antibotics.

The test system for inhibition of DNA and/or RNA synthesis in L1210 cells, which has been used for the study of structure–activity relationship at anthracycline antimetabolites[1,2], is adopted to the screening of nucleoside analogs. The series includes the 6-aza analogs of pyrimidine nucleosides, the studies of which started more than twenty years ago[3–6]. The structure–activity relationship is followed in more details at the derivatives of 5-fluorouracil and arabinosylcytosine, the known clinically used cancerostatic agents.

EXPERIMENTAL

The analogs tested were synthesized as indicated by references in Table I. Inhibition of nucleic acid synthesis in cultured leukemia L1210 cells was performed according to the procedure developed earlier[1,7,8]. L1210 cells were grown in RPMI 1640 medium containing 10% heat inactivated fetal bovine serum (FBS), 20 mM HEPES buffer at pH 7·2, 100 units/ml of penicillin, and 100 μg : : ml of streptomycin, and were maintained at a density of $1-4 \cdot 10^6$/ml. All compounds were tested for possible inhibition of the incorporation of [^{3}H]-thymidine into trichloroacetic acid precitable material. The compounds were weighed on a microbalance immediately before use and were dissolved in dimethyl sulfoxide. The above growth medium was added to yield a final dimethyl sulfoxide concentration of 2%. The analogs were assayed at final concentrations of 1 000, 100, 20, 10, 3, 1, 0·3, and 0·1 μmol l^{-1}. All groups of assays included a standard of daunorubicin as well as untreated control cells. One-half ml of L1210 cells at a concentration of $2 \cdot 10^6$/ml was added to 0·5 ml of the test solutions and incubated for 2 h at 37°C in a reciprocating shaker bath. Then, each assay of cells and test compounds was exposed for 1 h to 0·5 μCi/ml of [^{3}H]--thymidine (40 Ci/mol). and trichloroacetic acid precitable radioactivity was determined. The

* Part XL in the series Analogues of Nucleosides; Part XXXIX: This Journal *48*, 2088 (1983).

TABLE I

Inhibitory effect of nucleoside analogs *vs* nucleic acid synthesis in L1210 cells

Compound (ref.)	DNA[a]	RNA[a]
6-Azauracil[25]	>1 000	>1 000
1-Methyl-6-azauracil[25]	>1 000	>1 000
6-Azauridine[26]	229	810
5′-Methanesulfonyl-5-azauridine	>1 000	>1 000
O^2,2′-Cyclo-6-azauridine[12]	>1 000	>1 000
6-Azacytosine	>1 000	>1 000
6-Azacytidine[27,28]	>1 000	982
Arabinosyl-6-azacytosine[29]	>1 000	>1 000
6-Azaisocytosine	>1 000	>1 000
6-Azaisocytidine	>1 000	>1 000
Arabinosyl-6-azaisocytosine[12]	>1 000	>1 000
6-Azathymine[30]	>1 000	>1 000
4-Thio-6-azauracil	>1 000	>1 000
4-Thio-6-azauridine[27]	798	>1 000
2′,3′-Isopropylidene-4-thio-6-azauridine[27]	>1 000	>1 000
5′-Acetyl-2′,3′-isopropylidene-4-thio-6-azauridine[27]	>1 000	>1 000
Arabinosylurasil[31]	>1 000	>1 000
Arabinosylisocytosine[12]	>1 000	>1 000
Arabinosyl-4-thiouracil[31]	>1 000	>1 000
5-Fluorouracil	>1 000	55
5-Fluorouridine[26]	>1 000	0·9
5-Fluoro-2′-deoxyuridine[32]	200	58
5′-Deoxy-5-fluorouridine[33]	>1 000	615
2′,5′-Dideoxy-5-fluorouridine[34]	>1 000	>1 000
5′-Chloro-5-fluorouridine[33]	>1 000	>1 000
5′-Chloro-5-fluorocyclouridine[34]	>1 000	>1 000
Arabinosyl-5-fluorouracil	>1 000	>1 000
5′-Deoxyarabinosyl-5-fluorouracil[34]	>1 000	>1 000
Tetrahydrofuranyl-5-fluorouracil	>1 000	>1 000
Arabinosylcytosine[31,35]	0·13	>1 000
Tri-O-acetylarabinosylcytosine[36]	49	>1 000
N-Acetylarabinosylcytosine[37]	1·8	207
Tetraacetylarabinosylcytosine[38]	458	602
O^2,2′-Cyclocytidine.HCl[14]	0·3	37
5′-Chloroarabinosylcytosine[39]	4·4	>1 000
5′-Bromoarabinosylcytosine[22]	24[b]	
5′-Chlorocyclocytidine[39]	2·4[b]	
2′,5′-Anhydroarabinosylcytosine[22]	20[b]	
5′-Deoxyarabinosylcytosine[39]	>1 000	>1 000
5′-Chlorocytidine[39]	>1 000	288
5′-Deoxycytidine[39]	>1 000	146
5′-Deoxyadenosine[39]	516	278
5′-Chloroadenosine[39]	278	243
Daunorubicin	0·4	0·2

concentration producing a 50% inhibition of [^{3}H]-thymidine incorporation into trichloroacetic acid precitable material as compared with untreated controls (ED_{50}) was calculated for each compound. Appropriate concentrations in the range of the initial ED_{50} were selected for each compound, and assays were repeated. A similar technique using [^{3}H]-uridine provided ED_{50} values for inhibition of RNA synthesis.

RESULTS AND DISCUSSION

The 6-aza analogs were found very weak inhibitors of NA synthesis. 6-Azauridine inhibited both the DNA and RNA synthesis, 6-azacytidine was found a selective inhibitor of RNA synthesis. The difference between 6-azacytidine and 6-azauridine supported the original findings of their different mechanism of action[9-11]. The introducing of an appropriate secondary change into the original biologically active molecule, a change which could be potent in general to produce a biological activity, *e.g.*, to prepare 4-thio or arabinosyl derivatives, did not enhance the activity of the original molecule. Only 4-thio derivatives of 6-aza analogs expressed some activity while arabinosyl-4-thiouracil caused the greatest disappointment of our expectations.

An emphasized attention was devoted to a group of nucleoside analogs of a high chemical reactivity which should be able to interact with the biological system under the presentation of biological activity. A very fast and quantitative reaction of O^2,2'--cyclo-6-azauridine with ammonia[12] and/or aliphatic or aromatic amines[13] was observed but no biological activity was shown at these derivatives. A series of cyclonucleosides and epoxynucleosides were similarly found biologically not interesting. The known high activity of cyclocytidine[14] is an exception.

For a more detailed study on structure–activity relationship the derivatives of arabinosylcytosine and 5-fluorouracil were selected. The study was intended to find out to what extent the single functional groups retain the biological activity. A particular attention was devoted to the derivatives which were not able to form the 5'-phosphate esters, the proposed proper biologically active forms of these antimetabolites[15-17].

At the derivatives of 5-fluorouracil, the nucleobase was compared with its ribosyl and 2'-deoxyribosyl derivatives, and with the 5'-deoxy derivatives of both the ribo and the deoxyribo series, and with Ftorafur (1-((*R*, *S*)-tetrahydrofuran-2-yl)-5-fluorouracil, bearing no hydroxyl groups).

It was interesting to know if the derivatives would differ in their inhibitory effect *vs* synthesis of single types of nucleic acids. In contrast to a very strong activity

---

[a] Concentration in $\mu mol\ l^{-1}$ causing a 50% inhibition of nucleic acid synthesis; [b] concentration in $\mu mol\ l^{-1}$ causing a 50% inhibition of L1210 cell growth; the results were supplied by Dr A. Bloch from Roswell Park Memorial Institute; the comparative figures are for arabinocytosine 0·05, for cyclocytidine 0·08, and for 5'-chloroarabinosylcytosine 2·4.

of 5-fluorouracil *vs* RNA synthesis, Ftorafur did not inhibit either RNA or DNA synthesis. Thus, the C–N bond of Ftorafur is not cleaved under conditions of our test and, as the C–N bond of Ftorafur is hydrolysed faster than at deoxyribonucleosides[18], the deoxyribo and in particular the ribo nucleosides should pass the test without a cleavage of their nucleoside bond. 5-Fluorouridine expresses a specific inhibitory activity *vs* RNA synthesis almost by two order higher than 5-fluorouracil. At the 2',3'-isopropylidene derivative, the activity is expressively lowered similarly to the 5'-deoxy derivative of 5-fluorouridine. In contrary to that, the 5'-deoxy derivative of 2'-deoxy-5-fluorouridine does not exhibit any activity. 2'-Deoxy-5-fluorouridine, as the only one, exhibits a simultaneous effect *vs* DNA and RNA synthesis. We should therefore admit that the mechanism of its action differs from the other 5-fluorouracil derivatives. Also, the proposed transformation of 5-fluorouridine to 2'-deoxy-5-fluorouridine 5'-phosphate and following inhibition of thymidylate synthetase[15] should not be the only one mechanism of action for 5-fluorouridine. 5'-Chloro-5-fluorouridine does not exhibit any activity *vs* NA synthesis and 5'-deoxy--5-fluorouridine a very low one *vs* RNA synthesis. Both compounds were shown later on as promising prodrugs of 5-fluorouracil[19,20].

While the 5-fluorouracil derivatives belong to the most potent inhibitors of RNA synthesis the derivatives of arabinosylcytosine belong to the most potent inhibitors of DNA synthesis. Arabinosylcytosine itself exhibits the strongest specific inhibitory effect *vs* DNA synthesis. The specificity of action remains retained at tri-O-acetylarabinosylcytosine. Cyclocytidine expresses one of the most potent inhibitory effect *vs* DNA synthesis and simultaneously a very strong inhibition of RNA synthesis. We suppose that its biological action *in vivo* proceeds by another mechanism than only by its transformation to arabinosylcytosine. The N-acetyl derivative of arabinosylcytosine exhibits also a simultaneous effect *vs* DNA and RNA synthesis. It seems that the change of the 4-amino group to imino group or substitution of the amino group results in a change in the mechanism of action of the parent compound, arabinosylcytosine.

Out of the 5'-deoxy derivatives of arabinosylcytosine, 5'-chloroarabinosylcytosine exhibits the most surprising result with its very strong inhibition of DNA synthesis, while 5'-deoxyarabinosylcytosine is inactive. In constrast to arabinosylcytosine, 5'-chloroarabinosylcytosine is inactive[21] in inhibition of *Herpes simplex* virus. Originally, it was supposed that the 5'-chloro derivative is slowly hydrolysed to arabinosylcytosine which then expressed its biological activity. This suggestion was contradicted by further chemical[22] and biological[23] studies, where the 5'-chloro derivative was converted to the 2',5'-anhydro derivative of arabinosylcytosine[22]. Even if a partial conversion of the 5'-halogeno and of the 2',5'-anhydro derivatives to the biologically active arabinosylcytosine cannot be completely excluded, such a mechanism of action seems to be very unlikely. We may conclude that an interesting and promising new group of compounds of a strong biological activity has been found. The

attractiveness of these compounds is emphasized by the results on deamination by the isolated enzyme[23] and in particular by the results on penetration of nucleoside analogs through biomembrane[24].

If we look after the order of priority of the inhibitory effects separately *vs* DNA and/or RNA synthesis, the derivatives of arabinosylcytosine belong to the strongest inhibitors of DNA synthesis, in an order arabinosylcytosine, cyclocytidine, N-acetylarabinosylcytosine and the 5'-chloro derivative of arabinosylcytosine. 2'-Deoxy-5-fluorouridine is the only one representative of the 5-fluorouracil derivatives possessing inhibitory effect *vs* DNA synthesis. The most potent inhibitor of RNA synthesis is 5-fluorouridine while the second one is cyclocytidine. The activity of 5'-deoxy and/or 5'-chloro derivatives of cytidine and adenosine is a low one but the 5'-deoxy derivatives of adenosine belong to the rare nucleoside analogs which inhibit both the RNA and DNA synthesis. In this respect, cyclocytidine, as the only one, stands in its effectiveness very close to the most potent antibiotic cancerostatic agents.

In conclusion, we propose to accept the test system for inhibition of nucleic acid synthesis in L1210 cells as the first assay in search for new compounds promising cancerostatic activity. This test is able to find out the activity of the original, unchanged molecule, the results are obtained in a quantitative way and in an reproducible form, minimum supply of material is needed and, together with simplicity, it enables the scrrening of large series of compounds.

The authors express sincere thanks to Dr A. Bloch, Mrs D. L. Taylor, Dr H. Hřebabecký and Mrs J. Hlaváčková for a generous cooperation.

REFERENCES

1. Folkers K., Porter T. H., Acton E., Taylor D. L., Henry D.: Biochem. Biophys. Res. Commun. *83*, 353 (1978).
2. Acton E. M., Tong G. L., Mosher C. W., Smith T. H., Henry D. W.: J. Med. Chem. *22*, 922 (1979).
3. Gut J.: This Journal *23*, 1588 (1958).
4. Škoda J., Hess V. F., Šorm F.: Experientia *13*, 150 (1957).
5. Beránek J., Smrt J., Šorm F.: Czech. 96759. (1960).
6. Beránek J., Smrt J.: This Journal *25*, 2029 (1960).
7. Rusconi A., DiMarco A.: Cancer Res. *29*, 1507 (1969).
8. Meriwether W. D., Bachur N. R.: Cancer Res. *32*, 1137 (1972).
9. Novotný J., Smetana R., Rašková H.: Biochem. Pharmacol. *14*, 1537 (1965).
10. Handschumacher R. E., Škoda J., Šorm F.: This Journal *28*, 2983 (1963).
11. Škoda J., Šorm F.: Biochim. Biophys. Acta *91*, 352 (1964).
12. Beránek J., Šorm F.: This Journal *33*, 913 (1968).
13. Delia T. J., Beránek J.: Carbohydrates, Nucleosides, Nucleotides *4*, 349 (1977).
14. Beránek J., Brokeš J., Hřebabecký H.: 1502 Czech. (1977).
15. Cohen S. S., Flaks J. G., Barner H. D., Loeb M. R., Lichtenstein J.: Proc. Nat. Acad. Sci. U.S.A. *44*, 1004 (1958).
16. Ghu M. Y., Fischer J. A.: Biochem. Pharmacol *11*, 423 (1958).

17. Furth J. J., Cohen S. S.: Cancer Res. *28*, 2061 (1968).
18. Zhuk R. A., Berzinja A. E., Volynkina G. G., Hiller S. A.: Khim. Geterosikl. Soedin. *550* (1970).
19. Cook A. F., Holman M. J., Kramer M. J., Trown P. W.: J. Med. Chem. *22*, 1330 (1979).
20. Ajmera S., Danenberg P. V.: J. Med. Chem. *25*, 999 (1982).
21. Helgstrand E., Johansson N. G., Beránek J.: Unpublished results.
22. Hřebabecký H., Brokeš J., Beránek J.: This Journal *47*, 2961 (1982).
23. Kára J., Bártová M., Ryba M., Hřebabecký H., Brokeš J., Novotný L., Beránek J.: This Journal *47*, 2824 (1982).
24. Novotný L., Farghali H., Ryba M., Janků I., Beránek J.: Cancer Chemother. Pharmacol., *13*, 195 (1984).
25. Hřebabecký H., Beránek J.: This Journal *40*, 2364 (1975).
26. Beránek J., Hřebabecký H.: Nucleic Acids Res. *3*, 1387 (1976).
27. Beránek J., Šorm F.: This Journal *28*, 469 (1963).
28. Černěckij V., Chládek S., Šorm F., Smrt J.: This Journal *27*, 87 (1962).
29. Farkaš J., Beránek J., Šorm F.: This Journal *31*, 4002 (1966).
30. Beránek J., Gut J.: This Journal *34*, 2306 (1969).
31. Brokeš J., Beránek J.: This Journal *39*, 3100 (1974).
32. Brokeš J., Hřebabecký H., Beránek J.: This Journal *44*, 439 (1979).
33. Hřebabecký H., Beránek J.: Nucleic Acids Res. *5*, 1029 (1978).
34. Hřebabecký H., Beránek J.: This Journal *43*, 3268 (1978).
35. Beránek J., Brokeš J., Hřebabecký H.: Czech. 1501 (1977).
36. Beránek J., Drašar P.: This Journal *42*, 366 (1977).
37. Watanabe K. A., Fox J. J.: Angew. Chem. *78*, 589 (1966).
38. Martinez A. P., Lee W. W., Goodman L. J.: J. Med. Chem. *9*, 268 (1966).
39. Hřebabecký H., Brokeš J., Beránek J.: This Journal *45*, 599 (1980).

Translated by the author (J. B.).