PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 145, 542–545 (1974)

Antiviral Activity of 9-β-D-Arabinofuranosyladenine (ara-A) Against Gross Murine Leukemia Virus *in Vitro*[1] (37848)

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First synthesized as a potential anticancer agent in 1960 (1), 9-β-D-arabinofuranosyladenine (ara-A) has subsequently been shown to possess broad-spectrum activity against DNA-containing viruses in cell culture (2–12). In addition, significant therapeutic activity has been demonstrated by ara-A against herpesvirus types 1 and 2, equine abortion virus, and vaccinia virus infections *in vivo* (6, 13–21). Ara-A has also been shown to have significant antiviral activity against DNA virus infections in man, and therefore, the drug promises to have great practical utility in the treatment and clinical management of certain of these important viral diseases (22).

To date, only limited activity has been demonstrated for ara-A against RNA-containing viruses. An inhibitory effect of ara-A has been reported earlier on Rous sarcoma virus in cell culture (6, 7), and more recently, ara-A has been found to inhibit the replication of vesicular stomatitis virus *in vitro* (23). Because of these latter reports of ara-A activity against RNA viruses and because of the fact that the RNA tumor viruses replicate through a DNA intermediate made possible by the RNA-directed DNA polymerase (RDDP or "reverse transcriptase") reaction (24, 25), we have examined the effect of ara-A on naturally occurring Gross murine leukemia virus (MLV) *in vitro*.

Materials and Methods. Cell cultures and growth medium. Primary cell cultures prepared from 15- to 17-day-old NIH strain Swiss mouse embryos (NIH-ME) were obtained from Flow Laboratories, Inc. (Rockville, MD) and were used in the preparation of secondary cultures which were grown as monolayers in 60-mm plastic tissue culture dishes (Falcon Plastics, Inc., Oxnard, CA). The continuous-passage rat XC sarcoma cell line was obtained from Dr. Adeline J. Hackett (Naval Biomedical Research Laboratory, University of California, Berkeley, CA) and was carried in culture as monolayers in 75 cm^2 Falcon plastic tissue culture flasks. Eagle's minimum essential medium (MEM) with Earle's balanced salt solution (BSS) containing 10% unheated fetal calf serum, penicillin (100 units/ml), streptomycin (100 μg/ml), and glutamine (2 m*M*), was employed as growth medium for the propagation of both the NIH-ME cells and the rat XC cells.

Virus. The Gross MLV used in these studies was originally isolated from leukemic AKR mice and passaged five times in secondary NIH-ME cell cultures in these laboratories. The virus was identified as wild-type Gross MLV by means of a plaque-reduction neutralization test employing Gross MLV-specific antiserum. Virus stocks were prepared in secondary NIH-ME cell cultures, pretitered by means of the UV-XC plaque assay technique (26), and stored frozen at $-70°$ until used for experiments.

Compound. The ara-A (NSC 404241)

[1] This work was supported by Contract 71-2098 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare. We thank Mrs. Jo Ellen Jolley for excellent technical assistance.

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was supplied by Drug Research and Development, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

Plaque inhibition tests. The UV-XC plaque assay procedure for murine leukemia viruses, recently described by Rowe, Pugh, and Hartley (26), was utilized in the evaluation of ara-A for activity against Gross MLV by means of plaque inhibition tests. Five concentrations of ara-A ranging (in serial 0.5-$\log_{10}$ dilutions) from 40.0 to 0.4 μg/ml were examined for antiviral activity against 50 to 100 plaque-forming units (PFU) of Gross MLV/cell culture. Drug was added to the NIH-ME cell cultures immediately after virus inoculation. The test cultures (three cultures per drug concentration) each contained 4.5 ml of ara-A diluted in growth medium and 0.5 ml of virus suspension. Virus control cultures (six cultures) each contained 4.5 ml of growth medium alone (no drug) and 0.5 ml of the virus suspension. Drug control cultures (containing ara-A, but no virus) and normal cell control cultures (no drug or virus) were also included in each assay. All cultures were handled in a similar manner, incubated at 37°, and were quantitatively examined for Gross MLV infectivity by means of the UV-XC plaque assay technique at 96 hr after virus inoculation. Antiviral activity was expressed in terms of the reduction in the mean number of plaques counted in drug-treated, virus-infected cultures when compared with the mean number of plaques counted in untreated, virus-infected control cultures. Hemocytometer cell counts using trypan blue to determine viability were performed on triplicate drug-treated and untreated NIH-ME cell cultures at the time of virus assay.

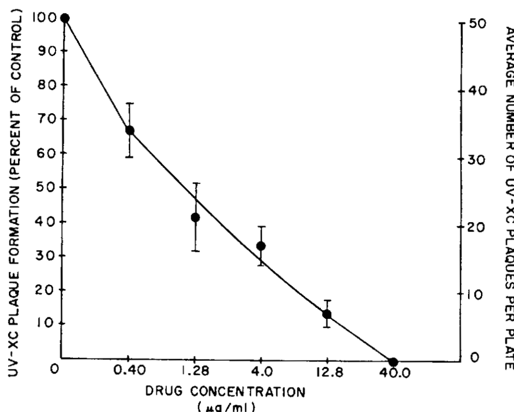

FIG. 1. Dose-dependent inhibition of Gross MLV-induced plaque formation in XC cells by ara-A (NSC 404241). Each point represents the average of triplicate assays (standard deviation shown).

Results. Data from a representative plaque inhibition test in which ara-A was evaluated for activity against Gross MLV *in vitro* are plotted in Fig. 1. (Six separate experiments yielded similar results.) It can be seen that ara-A significantly inhibited ($P < 0.001$) Gross MLV-induced plaque formation in XC cells at each of the five concentrations tested. A $\geq 50\%$ inhibition in the number of virus-induced plaques was obtained with as little as 1.28 μg of ara-A/ml of culture medium. An approximate 70% reduction in plaques was observed when virus-infected NIH-ME cells were treated with ara-A at a concentration of 4.0 μg/ml. No plaques were detected in UV-XC assays of virus-infected cultures treated with ara-A at a concentration of 40.0 μg/ml. Although there was no microscopic evidence of cytotoxicity at antiviral concentrations, Fig. 2 shows that the number of viable host cells which were present at 4 days after the initiation of drug treatment was slightly decreased in cultures treated with ara-A at 40.0 μg/ml. This indicated a possible inhibitory effect on host cell multiplication with higher concentrations of the compound. Miller *et al.* (7) reported a slight lowering of the mitotic index of H.Ep-2 cell cultures with ara-A at a concentration of 50 μg/ml, but not at lower concentrations. In these earlier studies, microscopic evidence of cytotoxicity was observed with the arabinosyl nucleoside at concentrations of 160 μg/ml and above, while selective antiviral activity was apparently demonstrated at concentrations of 40 μg/ml and below. Our data clearly demonstrated a selective antiviral effect for ara-A against Gross MLV at concentrations below 40 μg/ml, i.e., at concentrations of ara-A which produced a significant ($\geq 50\%$) inhibition of virus-

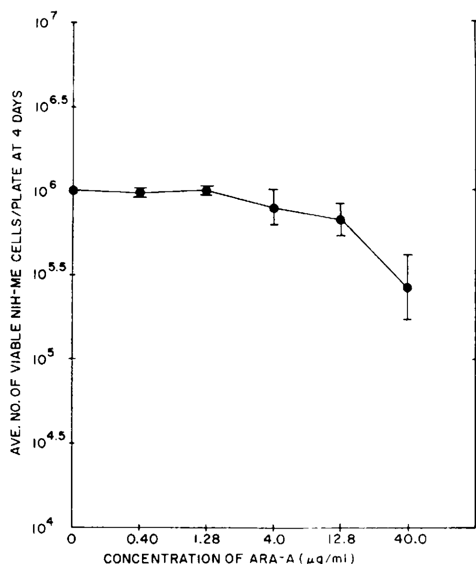

FIG. 2. Number of viable NIH-ME cells after treatment with ara-A for 4 days at 37°. Each point represents the average of triplicate assays (standard deviation shown).

induced plaque formation, but which had no demonstrable effect on the multiplication of host cells.

Discussion. The results of this investigation suggest that the idea of the restriction of ara-A antiviral activity to infections caused by DNA-containing viruses, as opposed to those caused by RNA-containing viruses, might have to be altered in the case of the RNA tumor viruses which have the capacity to produce virus-specific DNA using RNA as template. The search for selective antiviral agents which might be effective against oncogenic RNA viruses should therefore probably include an examination of potential inhibitors of both viral RNA and DNA synthesis. The inhibition of RNA-directed DNA polymerase from Rauscher murine leukemia virus by the 5′-triphosphate of the related compound 1-β-D-arabinofuranosylcytosine (cytosine arabinoside; ara-C), a well-known inhibitor of RNA tumor viruses and DNA viruses, was recently reported by Tuominen and Kenny (27). Similarly, Müller *et al.* (28) reported that the RNA-directed DNA polymerases from oncogenic RNA viruses and from lymphoma cells were approximately 200 times as sensitive to ara-CTP inhibition as the various viral and cellular DNA-directed DNA polymerases. Clearly, ara-A should also be examined for a possible selective inhibitory effect on viral RNA-directed DNA polymerases.

The practical importance of the finding of antiviral activity demonstrated by ara-A against Gross MLV, Rous sarcoma virus, and possibly other RNA tumor viruses *in vitro* cannot be accurately assessed until the compound has been thoroughly examined for possible confirmatory activity in experimental *in vivo* systems. Antiviral activity in cell culture does not necessarily indicate that one can expect to observe therapeutic effectiveness in animals or in man. However, the selective inhibition of Gross MLV by ara-A *in vitro* is a potentially important observation, and we are currently investigating the possibility of its similar inhibitory activity *in vivo*. The search for selective inhibitors of RNA tumor viruses could possibly lead to the development of useful chemotherapeutic agents for the prevention of virus-induced leukemia in animals and for the maintenance of remission in leukemic patients successfully treated with anticancer drugs or by other effective methods. An advantage of ara-A over ara-C therapy in human cancer patients would certainly be its demonstrated clinical effectiveness against DNA virus infections, which often appear as serious complications, and its apparent lack of immunosuppressive properties.

Summary. Ara-A, an antiviral agent with broad-spectrum activity against DNA viruses, was observed to inhibit Gross murine leukemia virus (an RNA tumor virus) in cell culture. Its activity was selective and was observed at concentrations well below cytotoxic levels.

1. Lee, W. W., Benitez, A., Goodman, L., and Baker, B. R., J. Amer. Chem. Soc. **82**, 2648 (1960).

2. De Garilhe, M. P., and De Rudder, J., C. R. Acad. Sci. **259**, 2725 (1964).

3. Freeman, G., Kuehn, A., and Sultanian, I., Cancer Res. **25**, 1609 (1965).

4. Freeman, G., Kuehn, A., and Sultanian, I.,

Ann. N. Y. Acad. Sci. **130**, 330 (1965).

5. De Rudder, J., and De Garilhe, M. P., Antimicrob. Ag. Chemother. **1965**, 578 (1966).
6. Schabel, F. M., Jr., Chemotherapy **13**, 321 (1968).
7. Miller, F. A., Dixon, G. J., Ehrlich, J., Sloan, B. J., and McLean, I. W., Jr., Antimicrob. Ag. Chemother. **1968**, 136 (1969).
8. Sidwell, R. W., Arnett, G., Dixon, G. J., and Schabel, F. M., Jr., Proc. Soc. Exp. Biol. Med. **131**, 1223 (1969).
9. Sidwell, R. W., Arnett, G., and Schabel, F. M., Jr., Progr. Antimicrob. Anticancer Chemother. **2**, 44 (1970).
10. Person, D. A., Sheridan, P. J., and Herrmann, E. C., Jr., Infec. Immunity **2**, 815 (1970).
11. Sidwell, R. W., Arnett, G., and Schabel, F. M., Jr., Chemotherapy **17**, 259 (1972).
12. Adamson, R. H., Ablashi, D. V., Armstrong, G. R., and Ellmore, N. W., Antimicrob. Ag. Chemother. **1**, 82 (1972).
13. Sidwell, R. W., Dixon, G. J., Sellers, S. M., and Schabel, F. M., Jr., Appl. Microbiol. **16**, 370 (1968).
14. Sidwell, R. W., Dixon, G. J., Schabel, F. M., Jr., and Kaump, D. H., Antimicrob. Ag. Chemother. **1968**, 148 (1969).
15. Schardein, J. L., and Sidwell, R. W., Antimicrob. Ag. Chemother **1968**, 155 (1969).
16. Sloan, B. J., Miller, F. A., Ehrlich, J., McLean, I. W., Jr., and Machamer, H. E., Antimicrob. Ag. Chemother. **1968**, 161 (1969).
17. Dixon, G. J., Sidwell, R. W., Miller, F. A., and Sloan, B. J., Antimicrob. Ag. Chemother. **1968**, 172 (1969).
18. Kaufman, H. E., Ellison, E. D., and Townsend, W. M., Arch. Ophthalmol. **84**, 783 (1970).
19. Lieberman, M., Pascale, A., Schafer, T. W., and Came, P. E., Antimicrob. Ag. Chemother. **1**, 143 (1972).
20. Allen, L., and Sidwell, R. W., Antimicrob. Ag. Chemother. **2**, 229 (1972).
21. Sloan, B. J., Miller, F. A., and McLean, I. W., Jr., Antimicrob. Ag. Chemother, **3**, 74 (1973).
22. Ch'ien, L. T., Schabel, F. M., Jr., and Alford, C. A., Jr., *in* "Selective Inhibition of Viral Function" (W. A. Carter, ed.), p. 227. Chem. Rubber Co. Press, Cleveland, OH (1973).
23. Grant, J. A., and Sabina, L. R., Antimicrob. Ag. Chemother. **2**, 201 (1972).
24. Temin, H. M., and Mizutani, S., Nature (London) **226**, 1211 (1970).
25. Baltimore, D., Nature (London) **226**, 1209 (1970).
26. Rowe, W. P., Pugh, W. E., and Hartley, J. W., Virology **42**, 1136 (1970).
27. Tuominen, F. W., and Kenny, F. T., Biochem. Biophys. Res. Commun. **48**, 1469 (1972).
28. Müller, W. E. G., Yamazaki, Z-I., Sögtrop, H. H., and Zahn, R. K., Eur. J. Cancer **8**, 421 (1972).

Received Sept. 24, 1973. P.S.E.B.M., 1974, Vol. 145.