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9-β-D-Arabinofuranosyladenine Inhibition of Chemically Induced Rat Embryo Cell Transformation (40326)

PAUL J. PRICE, P. C. SKEEN, AND C. M. HASSETT

Microbiological Associates' Torrey Pines Research Center, 2945 Science Park Road, La Jolla, California 92037

The antileukemic chemotherapeutic drug, 1-β-D-arabinofuranosylcytosine (ara-C) was previously shown to be an *in vitro* transforming agent (1) for an established line of Fischer rat embryo cells, which had previously been shown to be an accurate and sensitive indicator of chemicals having carcinogenic properties (2, 3). We were interested in using this same system to examine the transforming potential of 9-β-D-arabinofuranosyladenine (ara-A), an analog of ara-C which is also being used clinically as a cancer chemotherapeutic and antiviral agent in humans (4, 5). The antitumor and antiviral activities of both ara-A and ara-C appear to be derived from their inhibition of DNA synthesis (6–8). We report here that unlike ara-C, ara-A is not a transforming agent for Fischer rat embryo cells (F1706). Further, nontoxic levels of ara-A protect the cells from transformation induced by the known polycyclic hydrocarbon carcinogen, 3-methylcholanthrene (MCA).

Materials and methods. (A) Toxicity testing. Reduction in plating efficiency relative to a medium control was used to determine the toxicity of ara-A. Five hundred cells (F1706 D95) in 5 ml of the complete growth medium (Eagle's minimum essential medium in Earle's salts supplemented with 10% fetal bovine serum, 2 m*M* L-glutamine, 0.1 m*M* nonessential amino acids, 100 units of penicillin, and 100 μg of streptomycin/ml) were added to each 60-mm plastic cell culture dish (Lux). The dishes were incubated overnight at 37° in a humidified 5% CO_2-in-air incubator. The next morning the medium was decanted and replaced with a fresh medium containing serial dilutions of ara-A which had been diluted directly into the growth medium. Five days later the dishes were fixed and stained (methylene blue–carbol fuchsin) and macroscopic colonies were counted.

(b) Transformation assay. In two separate experiments run concurrently by two different investigators, F1706 D95 cells were inoculated into 75-cm^2 plastic cell culture flasks (Lux) at a concentration of 10,000 cells/ml and 14 ml per flask. On Days 2 and 5, cultures were refed with either growth medium alone or growth medium containing either 0.01 or 1.0 μg/ml ara-A. On Day 6, the cells from each group were transferred to fresh cultures in their respective media at a concentration of 1000 cells/ml and 10 ml per flask. The next day, 10 ml of growth medium was added to one-half the cultures from each group (without decanting the old media), and 10 ml of medium containing 0.4 μg/ml of MCA to the othe half. MCA was diluted in acetone to 1000 μg/ml and was further diluted in the growth medium. After an additional 2 days of incubation, the medium was decanted, and the cultures were washed with growth medium and refed with growth medium still supplemented with ara-A, but no longer containing the MCA. Three days later the cultures were again refed, but now with a growth medium void also of ara-A. The next day, new cultures were initiated at 500 cells/ml. This treatment schedule resulted in the following duplicate sets of cultures: media only (negative control), 0.2 μg/ml MCA (positive control), 0.01 μg/ml ara-A, 1.0 μg/ml ara-A, 0.01 μg/ml ara-A plus 0.2 μg/ml MCA, and 1.0 μg/ml ara-A plus 0.2 μg/ml MCA. At each subculture following the initial treatment, one set of flasks was set aside to be held without subdivision (holding series), and the other set subdivided 1:2 weekly to provide two new sets of cultures, one for the holding series and one for subdivision. Transformation was determined by the appearance of foci of cells lacking contact inhibition and orientation and by the formation of macroscopic colonies in semisolid agar (9). Tumorigenicity was determined by subcutaneous inoculation of 5×10^5 cells into newborn Fischer rats (F344/f Mai).

Results. We routinely test each compound for oncogenic potential at approximately the

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LD30 (concentration reducing the relative plating efficiency by approximately 30%) and at the highest concentration resulting in no reduction in relative plating efficiency (MNTD or maximum nontoxic dose). For ara-A these levels were 1.0 and 0.01 μg/ml, respectively (Table I).

At neither level did ara-A, itself, induce cell transformation of F1706 cells. However, as expected, cells treated with 0.2 μg/ml MCA were phenotypically transformed by the third vertical subculture (D + 3), and when tested at D + 6 produced macroscopic colonies in semisolid agar. When tested at D + 3, all cultures were negative for growth in agar. Cultures treated with MCA in the presence of either level of ara-A were still phenotypically normal at the termination of the experiment 8 subcultures after treatment and failed to grow in semisolid agar when tested at D + 3 and D + 6. When inoculated into the newborn Fischer rats at D + 8, the cultures treated with MCA alone were tumorigenic. The first tumor was found 52 days postinoculation and by the 82nd day, 11 of the 14 rats were positive. In contrast, a total of 45 rats inoculated with cells from cultures treated 8 subcultures earlier with either ara-A or MCA in the presence of ara-A were still tumor free when the experiment was terminated 94 days postinoculation (Table II).

TABLE I. TOXICITY OF ara-A[a] AS DETERMINED BY REDUCTION IN PLATING EFFICIENCY OF F1706 D95[b].

Concentration (μg/ml)	Relative plating efficiency[c] (%)
100	21
10	45
1.0	73
0.1	87
0.01	95
0.001	100

[a] 9-β-D-Arabinofuranosyladenine.

[b] A serial line of Fischer rat embryo cells in its 95th population doubling.

[c] The percentage of cells giving rise to macroscopic colonies, relative to the media only control, in which the absolute plating efficiency was arbitrarily set at 100%. The absolute plating efficiency of the control was 20% (108 colonies out of 500 cells plated).

Discussion. Many drugs used in cancer chemotherapy are transforming agents (1, 10, 11), mutagens (12), and oncogens (13, 14). One such agent, ara-C, had previously been found to induce transformation in mass cultures of secondary hamster embryo cells (15). This observation was later confirmed using a quantitative hamster transformation system, as well as the F1706 cells used in the present study (1). Subsequently, it was demonstrated,

TABLE II. MCA[a]-INDUCED TRANSFORMATION OF F1706[b] AND PROTECTION FROM TRANSFORMATION BY ara-A.

Treatment (per ml)	Morphological transformation[c]	Growth in agar (D6)[d]	Tumor results,[c] No. positive/No. inoculated (days to 1st tumor–days to last tumor)
Media control	− (+8)	−	ND[e]
Media control	− (+8)	−	0/5
0.2 μg/ml MCA	+ (+3)	+	11/12 (56–82)
0.2 μg/ml MCA	+ (+3)	+	0/2[f]
1.0 μg ara-A	− (+8)	−	0/9
1.0 μg ara-A	− (+8)	−	0/13
0.01 μg ara-A	− (+8)	−	ND
0.01 μg ara-A	− (+8)	−	ND
1.0 μg ara-A + 0.2 μg MCA	− (+8)	−	0/10
1.0 μg ara-A + 0.2 μg MCA	− (+8)	−	0/13
0.01 μg ara-A + 0.2 μg MCA	− (+8)	−	ND
0.01 μg ara-A + 0.2 μg MCA	− (+8)	−	ND

[a] 3-Methylcholanthrene.

[b] A serial line of Fischer rat embryo cells.

[c] Newborn Fischer rats inoculated with 5×10^5 cells (0.05 ml) from D + 8. Rats without tumors were held 94 days and then sacrificed.

[d] Triplicate agar dishes were each inoculated with 50,000 cells from cultures at D + 6 (6 population doublings after removal of the MCA), held 4 weeks at 37° in a humidified 5% CO_2 incubator, and screened for the appearance of macroscopic colonies.

[e] Not done.

[f] Twelve rats inoculated, 10 killed by mother.

using the $C_3H/10T1/2$ mouse embryo cells (16), that oncogenic transformation took place maximally in the S phase of the cell cycle (17). We know from double-blind studies that 90% of the chemicals which transform these cells are also oncogenic for mice and rats (2). Since it is possible that tumor induction in the rodent may be relevant to tumor induction in man, it seems wise to avoid where possible the use of chemotherapeutic agents which transform rodent cells. Ara-C is a transforming agent. Ara-A did not transform the F1706 rat cells, and at nontoxic doses protected the cells from transformation induced by the potent carcinogen, MCA.

We have previously used this *in vitro* system (F1706) to show that several antiviral antibiotics, i.e., streptonigrin (18), cordycepin (19), and geldanamycin (20), could protect the cells from chemically induced transformation. We suggested that this protection was due to the ability of the antibiotic to inhibit endogenous oncorna virus expression, since each drug also inhibited the "turn-on" of endogenous virus by halogenated pyrimidines. This explanation, however, is not applicable to ara-A protection of MCA-induced cell transformation, since ara-A did not inhibit transient virus induction by halogenated pyrimidines under similar conditions.

These studies suggest that *in vitro* cell transformation assays may have value, not only as a prescreen for potentially oncogenic chemicals, but also for compounds having anticancer properties.

Summary. The cancer chemotherapeutic and antiviral agent 9-β-D-arabinofuranosyladenine (ara-A) was examined for potential oncogenicity, using a serial line of Fischer rat embryo cells, which was previously shown to be a sensitive and accurate indicator of chemicals carcinogenic for rodents. We report here that at the concentrations tested, ara-A was not a transforming agent. Further, ara-A protected the cells from transformation induced by the known carcinogen, 3-methylcholanthrene.

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