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SHORT COMMUNICATIONS

Failure of 5-ethyl-2′-deoxyuridine to induce oncogenic RNA (oncorna) viruses in Fischer rat embryo cells and in Balb/3T3 mouse cells

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Genetic information for the synthesis of C-type RNA tumor viruses is present in the host genome of vertebrates in a repressed form [1, 2]. Several chemicals have the potential of activating such viruses. These include certain corticosteroids [3], inhibitors of protein synthesis [4] and some analogues of the natural pyrimidine bases. Among the base analogues the derivatives 5-iodo-2′-deoxyuridine (IdUrd) and 5-bromo-2′-deoxyuridine (BrdUrd) have been studied in detail [5–8]. Incorporation of the 5-iodo and 5-bromouracil into the cellular DNA seems to play an important role in virus activation [9, 10].

Since the uracil moiety of the virostatic 5-ethyl-2′-deoxyuridine [11] (EtdUrd) also readily enters the DNA of vertebrate cells under *in vitro* [12, 13] and *in vivo* [14] conditions, it was of interest to study the inductive potential of this compound in activating RNA (oncorna) viruses.

The EtdUrd was a pure sample of the β-anomer having a melting point of 177–179°. IdUrd, included for the sake of comparison, was obtained from Sigma Chemical Company, U.S.A.

Induction studies were performed in Fisher rat embryo cells (F-1706) and in non-producing (NP) Balb/3T3 cells infected with the Kirsten strain of murine sarcoma virus (Ki-MSV). Those were designated as Balb/K cells [15]. The cells were cultured in plastic T-75 flasks in Eagle's minimal essential medium with 10% fetal calf serum, 2 mM L-glutamine and a penicilline–streptomycin mixture at 100 μg/ml.

The virus activated from the rat cells was determined by the reverse transcriptase test alone, but the infectious one from the Balb/K cells was determined both by the reverse transcriptase and by the sarcoma virus focus assay on normal rat kidney (NRK) cells. The reverse transcriptase assay was carried out on tissue culture fluids that were clarified at a low speed, pelleted at 39,000 rev/min for 1 hr in the I.E.C. model B-60 ultracentrifuge and suspended in 0.2 ml of 0.01 M Tris buffer pH 8.0. The assay was performed in a total volume of 100 μl containing 40 μl of suspended viral pellet, 0.1% Nonidet-P 40 (Shell Co., United Kingdom), 0.006 units at A_{260} of the synthetic heteropolymer poly r (A). oligo d $(T)_{12-18}$ (Collaborative Research, U.S.A.), 40 mM Tris–HCl (pH 8.3), 5 mM dithiothreitol, 30 mM NaCl, 0.6 mM $MnCl_2$ and 6 μCi of (^{3}H) deoxythymidine-5′-triphosphate (40–50 Ci/m-mole, Amersham/Searle Corp.). The reaction mixture was incubated for 1 hr at 37° and at the end of the incubation period was spotted on Whatman DE-81 cellulose filter disks [16]. The filters were dried and washed six times with 5% Na_2HPO_4 and three times with distilled water, dried and counted in 10 ml toluene based scintillation fluid. Three cell cultures induced by IdUrd were labelled by ^{3}H-uridine (20 μCi per ml) for 24 hr. These fluids were pelleted and used for centrifugation in isopycnic sucrose gradients.

The results with EtdUrd and IdUrd in Fischer rat cells are presented in Table 1. In these cells EtdUrd was inactive at 20 and 50 μg/ml, whereas IdUrd was active at 20 μg/ml. With IdUrd the reverse transcriptase activity increased up to 120 hr after the removal of the chemical but declined subsequently. A primary increase and a subsequent loss of the transcriptase activity in transformed cell lines has been reported [17].

In Balb/K cells also (Table 2) EtdUrd was incapable of inducing the viral reverse transcriptase. A virus check, by the sarcoma foci formed on NRK cells, was negative. Whereas at 20 μg/ml IdUrd induced the transcriptase only at 40 μg virus could also be detected by both the enzymic and the sarcoma focus assay. In an isopycnic sucrose gradient the virus banded around 1.16 g/ml which is the density of the C-type virus [18] and this band corresponded to the peak in reverse transcriptase activity in the same gradient. Attempts to inhibit the IdUrd directed virus induction by simultaneous treatment with a double molar EtdUrd in both the rat and the mouse cell lines were unsuccessful. (Table 3).

Table 1. Viral transcriptase activity in Fischer rat embryo cells as a function of 5-ethyl-2′deoxyuridine (EtdUrd) and 5-iodo-2′-deoxyuridine (IdUrd)

Time (hr)	Viral transcriptase: Control	IdUrd 20 μg/ml	EtdUrd 20 μg/ml	EtdUrd 50 μg/ml
0	178	435	145	150
48	215	466	173	162
120	159	1,501	168	184
168	158	1,380	159	142

The viral transcriptase activity is expressed in cpm of ^{3}H-TMP incorporated per ml of tissue culture fluids. One ml of culture fluid is equivalent to 2×10^7 cells. See text for the experimental details.

Table 2. Effect of the EtdUrd and IdUrd on the induction of C-type particles in Balb/K cells

Treatment*	Reverse transcriptase†	No. of foci†
40 μg IdUrd/ml	35,410	1.9×10^2
5 μg EtdUrd/ml	533	0
20 μg EtdUrd/ml	641	0
50 μg EtdUrd/ml	610	0
None	617	0

* Cells were treated with the deoxyuridine for 72 hr.

† The reverse transcriptase activity (in cpm of ^{3}H-TMP incorporated) and the No. of foci are expressed per ml (2×10^7 cells) of tissue culture fluid.

* Dedicated to Professor Dr. Wilhelm Friedrich on his 60th birthday.

Table 3. Inability of 5-ethyl-2′-deoxyuridine (EtdUrd) to block the induction of C-type virus in mouse and rat cells by 5-iodo-2′-deoxyuridine (IdUrd)*

Cells	Treatment	No. foci on NRK cells/ml of tissue culture fluids	cpm of ^{3}H-TMP incorporated/ml of tissue culture fluid†
Mouse Balb/K Cells	IdUrd 20 μg/ml	1.6×10^2	94,982
	IdUrd 20 μg/ml EtdUrd 50 μg/ml (24 hr prior to IdUrd treatment)	1.73×10^2	76,230
	None	—	930
Fischer rat embryo cells	IdUrd 20 μg/ml	—	1,130
	IdUrd 20 μg/ml EtdUrd 50 μg/ml (22 hr prior to IdUrd treatment)	—	1,171
	None	—	201

* Cultures were treated with the deoxyribosides for 72 hr.
† Average of 3 determinations.

However, Teich *et al.* [9], reported that at equimolar concentrations *d*-thymidine completely inhibited the inductive effect of IdUrd in AKR mouse cell line.

Since EtdUrd also fails to activate the virus in the highly inducible AKR cells, it is likely that in general, EtdUrd does not act as an activator of oncorna viruses.

For the clinical application of EtdUrd these results are of particular importance, because evidence has indicated that chemically induced oncorna viruses, especially as shown for IdUrd, ultimately lead to carcinogenesis *in vivo* [19].

University Eye-Clinic, KAILASH K. GAURI
2 Hamburg 20,
Federal Republic of Germany.

Microbiological Associates, ILAN SHIF
Bethesda, Md.

National Cancer Institute RONALD G. WOLFORD
Bethesda, Md. U.S.A.

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