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PRELIMINARY COMMUNICATIONS

ON THE MODE OF ACTION OF 5-VINYL-2'-DEOXYURIDINE

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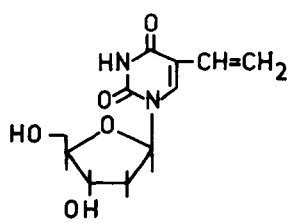
In a recent paper we reported the inhibitory effect of 5-vinyl-2'-deoxyuridine (vin-UdR) on the multiplication of Ehrlich ascites carcinoma cells in culture[1]. The effect can be prevented by the addition of thymidine in concentrations of about 1/10 of that of the inhibitor. In the following we describe results from which we conclude that inhibition by vin-UdR is due to incorporation of the analogue into DNA.



Methods

For the estimation of DNA synthesis, 10 mg Ehrlich ascites carcinoma cells were incubated for 1 hour in 0.3 ml Ringer solution supplemented with glucose and bicarbonate in the presence of either ^{32}P-phosphate (10 μCi, carrier free) or ^{3}H-thymidine (3 μCi, spec. act. = 10 Ci/mmole), and analyzed as described earlier[2].

5-Vinyl-2'-deoxyuridine was synthesized from acetylated 5-ethyl-2'-deoxyuridine (a generous gift from Dr. Vorbrüggen, Schering AG, Berlin-West) by bromination with equimolar amounts of bromine or N-bromosuccinimide in tetrachloromethane under UV-catalysis

to give acetylated 5-(α-bromo)ethyl-2'-deoxyuridine, followed by dehydrobromination with N-ethyldiisopropylamine. F: 155°C. MS (MS 902 AEI Manchester): Molpeak 254,0915, calcd. for $C_{11}H_{14}N_2O_5$. Elemental analysis: Calcd. C, 52.01; H, 5.55; N, 11.02;

Found C, 52.02; H, 5.49; N, 11.01.

Ultraviolet absorption properties: λ_{max} (methanol) 290 nm (ε = 8.400), and 235 (ε = 11.000).

Results

In table 1 the effect of the compound on cell multiplication and on ^{32}P-phosphate or ^{3}H-thymidine incorporation is described. It follows that at concentrations 10 times higher than necessary for a 50% inhibition of cell multiplication, there is no inhibition of DNA synthesis as measured by ^{32}P-phosphate incorporation. In fact, an increase was found, which is in agreement with the enhancing effect of thymidine and thymidine analogues in this system[3]. This is in sharp contrast to the effects of other antimetabolites in this system, such as 5-fluorouracil, cytosinearabinoside, folic acid antagonists and 5'-fluorothymidine[2]. All these compounds interfere either with the synthesis of precursors, especially of thymidine 5'-triphosphate, for DNA synthesis or - as cytosine arabinoside - directly inhibit DNA polymerase. Both modes of action find their immediate expression in the rate of DNA synthesis as measured by ^{32}P-phosphate incorporation. It follows from the results mentioned above that vin-UdR, in contrast to these compounds, does inhibit neither precursor synthesis nor DNA polymerase. In addition, vin-UdR abolishes the inhibitory effect of 5-fluoro-2'-deoxyuridine. In this respect the effect of the compound resembles that of thymidine[3].

Quite in contrast to ^{32}P-phosphate incorporation, there is an immediate inhibition of ^{3}H-thymidine incorporation into DNA; this suggests that there must be an effective competition of vin-UdR with thymidine

TABLE 1. Influence of 5-vinyl-2'-deoxyuridine and 5-ethyl-2'-deoxyuridine on multiplication and DNA synthesis of Ehrlich ascites carcinoma cells (as measured by cell count and ^{32}P-phosphate or ^{3}H-thymidine incorporation). Cell multiplication was determined after 24 hours of incubation in suspension culture[4]. The cell number in the controls increased 2.1 to 2.3 fold. Data were taken from[1]. DNA synthesis was estimated after 1 hour of incubation with the labelled precursors. Control samples contained 1025 cpm or 23023 cpm of incorporated ^{32}P-phosphate and ^{3}H-thymidine, respectively.

	% Inhibition of			
	Multiplication in cell culture	Incorporation (into DNA) of ^{32}P-phosphate		^{3}H-thymidine
Inhibitor Concentration (mM)	0.1	0.01	1.0	1.0
5-Vinyl-2'-deoxyuridine	55		0 (85% increase)	82
5-Ethyl-2'-deoxyuridine	19		0 (85% increase)	71
5-Fluoro-2'-deoxyuridine		54		
5-Fluoro-2'-deoxyuridine + 5-Vinyl-2'-deoxyuridine		0 (46% increase)		

incorporation into DNA reflecting, at the biochemical level, the antagonism between both compounds observed earlier with regard to cell multiplication. In our opinion these results can be explained best by an incorporation of vin-UdR into DNA, which does not put an immediate stop to DNA synthesis but causes inhibition of this process later on. The action of vin-UdR would be similar in this respect to that of 5-ethyl-2'-deoxyuridine (included in the table for comparison)

which is also incorporated into DNA of mammalian cells[5]. As the results show, 5-ethyl-2'-deoxyuridine is, however, somewhat less cytotoxic than vin-UdR. It cannot yet be excluded definitely that inhibition of thymidine incorporation is due to inhibition of some other step in the incorporation of exogeneous thymidine, e.g. its uptake or phosphorylation by thymidine kinase. Since these inhibitions would hardly affect cell multiplication, one would then have to look for an inhibitory effect of the compound in a metabolic pathway other than DNA synthesis. This is, however, much less likely because both the structure of the compound and the prevention of its inhibitory effect by thymidine suggest strongly DNA replication as being the target of vin-UdR. The free base 5-vinyluracil was recently found by Chelton, Evans, Jones and Walker[6] to be incorporated into the DNA of thymine requiring mutants of E.coli.

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