EFFECT OF 5-FLUOROURACIL AND 5-BROMO-2′-DEOXYURIDINE ON THE INDUCTION OF THYMIDINE KINASE IN POLYOMA VIRUS-INFECTED MOUSE EMBRYO CELLS

Jaroslav Žemla

Institute of Virology, Czechoslovak Academy of Sciences

Bratislava, Mlynská dolina, Czechoslovakia

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It has been demonstrated that polyoma virus induces in mouse cells thymidine (dTR) kinase (Frearson et al., 1965; Dulbecco et al., 1965; Sheinin, 1965; Hartwell et al., 1965; Kit et al., 1966; Kára and Weil, 1967) which is synthesized de novo (Kit et al., 1966) and some of its properties differ from those of the host enzyme (Sheinin, 1966; Ricceri and Cocuzza, 1967). Besides that, polyoma virus stimulates under certain conditions also the cellular DNA synthesis which appears essential for virus reproduction (Basilico et al., 1966; Ben Porat et al., 1966).

In this work we raised the following questions : (1) Does the induction of dTR kinase proceed also in cells with inhibited virus reproduction as a result of application of 5-fluorouracil (FU) and 5-bromo-2′-deoxyuridine (BUDR) (Ben Porat et al., 1966; Smith et al., 1966) ? (2) If the enzyme is induced, what is its kinetics, namely in relation to a possible modification of viral and cellular DNA by the incorporated BUDR ? The present results show that dTR kinase is induced in the absence of a normal reproduction of infective virus, however, the peak enzyme activity is lower and both its increase and decre-

ase are considerably delayed as compared to the normal infection.

MATERIALS AND METHODS

4-5 days old primocultures of mouse embryo cells in 10 cm Petri dishes were pretreated for 24 hours with FU (10 ug/ml) in the fresh medium (No.199 with 10 % of horse serum), then infected with 2-20 PFU per cell of polyoma SE virus during 3 hours. After removing the virus inoculum cells were incubated with FU (10 ug/ml) plus BUDR (100 ug/ml) in the fresh medium for 3 days at 37°. FU was added to the cells to increase the incorporation of BUDR into DNA (Kamiya et al.,1964,1965). Similarly treated,but "mock-infected" cultures served as controls. In another control series FU and BUDR were omitted. Crude extracts were prepared from duplicate cultures in daily intervals and the specific activity of dTR kinase was estimated using dTR-H^3 (ÚVVVR,Prague) as substrate (Kamiya et al.,1965). The scintillation counting was performed in the Nuclear Chicago spectrometer Model 6801. Proteins were assayed by the method of Lowry et al. (1951).

RESULTS AND DISCUSSION

The results of one representative experiment are shown in Table 1. Under the conditions of a normal infection dTR kinase culminated 24 hours post infection (p.i.), when reached 263.8 % of the control before infection.Thereafter it decreased sharply to zero to 72 hours p.i. Since the enzyme activity in the non-infected cells underwent also certain variation and finally dropped to negligible values due to the aging of cultures, the relative enzyme induction - expressed in virus/control % ratios - possessed a particular characteristics.

In FU-pretreated cells which exerted a considerably lower

Table 1.

EFFECT OF FU AND BUDR ON THE INDUCTION OF dTR KINASE IN POLYOMA VIRUS-INFECTED MOUSE EMBRYO CELLS

Sample	Days p.i.	uumoles dTMP /mg protein /30 min.	% ratios		
			Time course	Virus/ control	B/A
Control	0	733.7	100.0		
	1	941.8	128.4		
	2	53.5	7.3		
	3	45.2	6.2		
Virus (A)	1	1935.5	263.8	205.5	
	2	536.4	73.1	1002.6	
	3	0	0	0	
Control FU/FU+BUDR[&]	0	281.5	100.0		
	1	476.5	169.3		
	2	244.2	86.7		
	3	647.0	229.8		
Virus (B) FU/FU+BUDR[&]	1	1123.4	399.1	235.8	58.0
	2	1406.7	499.7	576.0	262.2
	3	1007.1	357.8	155.7	/∞/

[&] FU (10 ug/ml) present 24 hours before infection ; FU (10 ug/ml) plus BUDR (100 ug/ml) present after infection.

"starting" enzyme activity the infection in the presence of FU plus BUDR caused also an induction. However, its peak was lower and delayed to 48 hours p.i., and its fall proceeded more slowly as compared to the normal infection. The changed kinetics of the enzyme induction is demonstrated namely in the relative %

ratios of enzyme activities (see B/A column, Table 1) which show values of 58.0 - 262.2 -/∞/ at the times of 24 - 48 - 72 hours p.i., respectively.

Our results with BUDR show - in accordance with those of Kit et al. (1966) - that dTR kinase may be induced in mouse embryo cells by polyoma virus in the absence of a normal reproduction of infective virus. The changed intensity and kinetics of the enzyme induction in the presence of BUDR plus FU may be explained in several ways, e.g. (1) control system for dTR kinase is impaired due to the malfunction of BUDR-containing viral and/or cellular genes ; similar effect of BUDR was observed in the case of pseudorabies virus (Kamiya et al.,1964,1965), namely the absence of the normal cut off of several virus-induced activities. (2) The incorporation of FU into messenger RNAs may cause similar effects as mentioned under (1) due to the formation of nonfunctional proteins (Horowitz et al.,1960). (3) FU being incorporated into RNA inhibits irreversibly the cellular (Rich et al.,1958; Kaplan and Ben Porat,1961) as well as the polyoma virus DNA (Ben Porat et al.,1966) synthesis. It is not known whether this block of DNA synthesis is relieved by BUDR or not.In any case the delayed and partially depressed dTR kinase induction may reflect a generally partially inhibited and slowed viral DNA formation. In this connection it should be mentioned that Ben Porat et al. (1966) did not find an induction of dTR kinase in this virus-cell system treated with FU alone.

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