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THE ROLE OF CELL TYPE IN TRANSPORT AND METABOLIC CONVERSION OF ANTIVIRAL SUBSTANCES

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Summary. — The transport and metabolic conversion of 6-azauridine differed when compared in HeLa and chick embryo (CE) cells. The values of 9-(S)-(2, 3-dihydroxypropyl) adenine transported into the cells were found different for ZP cells (rabbit lung cell line), HeLa and CE cells. These differences were less expressed if relating the values of cellular uptake and metabolic conversion to the cell volume of the respective cell type. The differences seem to play a role in quantitation of the antiviral potency of the compounds in different host cells.

Key words: inhibition; virus replication; cell culture; 6-azauridine

Reports both from the early phase of the antiviral research as well as the recent ones have described differences in the antiviral potency of substances against the same virus when compared in different cell cultures. In various host cells the virus inhibitory concentration was different. Appleyard *et al.* (1965) studied the inhibition of rabbit pox virus by isatin-β-thiosemicarbazone (IBT) in different cell lines. Among the cells tested, those of primate origin appeared to be the most sensitive to inhibition of the virus growth by IBT. Inhibition by IBT was about 100 times less effective in rabbit kidney RK_{13} and L cells than in HeLa cells. In our laboratory we have determined that ten-fold higher concentration of 6-azauridine was necessary for 99 per cent inhibition of vaccinia virus in CE cells than in HeLa cells (Rada and Blaškovič, 1961; 1966). Similarly, Person *et al.* (1970) found differences in sensitivities of 21 strains of herpes simplex virus to 5-iodo-2′-deoxyuridine and 9-β-arabinosyladenine when evaluated in CE cells, WI-38 (human foetal diploid fibroblast cell strain) and HeLa cell cultures. This phenomenon was studied in more detail by De Clercq (1982). He compared the inhibitory effect of seven inhibitors of herpes simplex virus replication in altogether 12 cells of human, simian, feline and murine origin. The 50 per cent inhibitory doses of the compounds varied considerably from one cell line to another. Similarly, differences in the antiviral potency of acycloguanosine against herpes simplex virus type 1 were found when tested in different cell cultures (Harmenberg *et al.*, 1980; Harmenberg and Wahren, 1982). Recently, variation in sensitivity of herpes simplex type 1 virus in two different cell lines was also observed

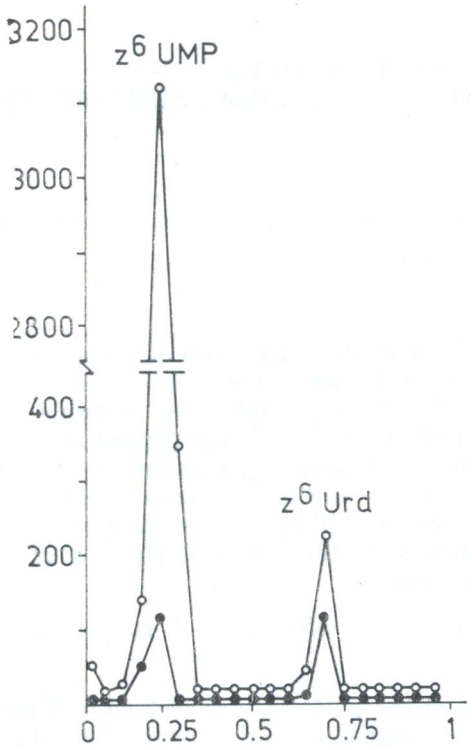

Fig. 1.
Transport and metabolic conversion of 6-azauridine in HeLa and CE cells
●——● chick embryo cells (Σc.p.m. 302)
○——○ HeLa cells (Σ c.p.m. 4003)
Abscissa: R_F values; ordinate: c.p.m. per 2×10^6 cells

with some novel analogues of (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (Reefschläger *et al.*, 1984).

No explanation is readily apparent for this variation in the drug sensitivity of the virus in different cell culture systems. The present model study using the antiviral substance 6-azauridine (z⁶Urd) will show that two factors — the transport into the cell and the metabolic conversion of the nucleoside analogue — may play a role in this phenomenon.

Suspension cultures of HeLa and CE cells were supplemented with ^{14}C-6-azauridine (19 kBq/ml). After 1 hr incubation the cells were washed and the cell pellet was precipitated by trichloracetic acid. The respective supernatants were analysed by paper chromatography in isopropanol-ammonium hydroxide-H_2O (7 :1 : 2, v/v/v). The sum of c.p.m. in CE cells was 302; in HeLa cells 4003. (The concentration of ^{14}C-6-azauridine added was 6×10^5 c.p.m./ml). Compared with the amount of z⁶Urd added, it can be seen (Fig. 1) that 0.05 per cent of z⁶Urd was transported into CE cells, in comparison with 0.7 per cent of z⁶Urd transported into HeLa cells. Out of the amount of z⁶Urd which was transported into the cells, 48 per cent and 89 per cent were converted to monophosphate (the effective final inhibitor) in CE and HeLa cells, respectively.

Table 1. Characteristics of 6-azauridine transport into CE, HeLa and L cells

Cells	Chick embryo	HeLa	L
Per cent of z^6Urd per 2×10^6 cells	0.06	0.7	0.8
Cell volume ($\mu l/10^6$ cells)	1.5	4.4	3.5
$\frac{z^6Urdi^*}{z^6Urde}$	0.2	0.8	1.1

* The ratio of intracellular and extracellular z^6Urd equilibrium concentrations (intracellular concentration of z^6Urd after two washes of the cells was calculated from specific cell radioactivity related to the cell volume, extracellular concentration of z^6Urd is represented by that added to the cells).

However, if these values were related to the cell volume, the differences in the intracellular concentrations were smaller. The ratio of intracellular and extracellular z ^{6}Urd concentration is given in Table 1. To determine the cell volume, a definite number of cells in supension ($200-500 \times 10^6$ cells) was centrifuged. The pellet was resuspended in an exact volume of cold saline and the cell suspension was measured as a whole. The cell volume was estimated from the difference of both volumes based on the fact that extracellular space in the cell pellet does not exceed 15 per cent of the cell pellet volume (Plagemann *et al.*, 1978). Comparison of the actual intracellular concentrations (i.e. related to the cell volume) of 6-azauridine monophosphate in CE and HeLa cells showed that these were similar even when the concentration of the analogue in the extracellular medium differed ten-fold (Table 2). This ten-fold concentration difference was required to cause the same degree of virus inhibition in the two cell types (Rada and Blaškovič, 1961; 1966). Furthermore, the level of uridine kinase in HeLa (and also L) cells was more than ten-fold higher as compared with CE cells (Rada, 1970).

Our previous studies have shown that, in contrast to the most nucleoside analogues, 9-(S)-(2,3-dihydroxypropyl)adenine (DHPA) was metabolized neither in uninfected nor in virus-infected cells (Rada *et al.*, 1980). Further studies on the transport of DHPA into different cell types have shown differences in the uptake of this analogue. The maximum values of tranported

Table 2. Intracellular concentration of 6-azauridine monophospnate in CE cells and HeLa cells

Extracelular concentration of z^6Urd (mM)*	6-Azauridine monophosphate in Chick embryo cells nmol/10^6 cells mMi**		HeLa cells nmol/10^6 cells mMi**	
1			2.56	0.58
10	0.81	0.54		

* Extracellular concentrations of z^6Urd are those added to the cells.

** Intracellular concentration of z^6UMP was calculated from specific cell radioactivity of z^6UMP related to the cell volume.

DHPA in equilibrium were observed in ZP cells (about 40 pmoles per 10^6 cells). In HeLa cells the values varied in the range of 15—25 pmoles per 10^6 cells. Into CE cells DHPA was transported at 3 to 10-fold lower eficiency than to ZP cells (4—15 pmoles per 10^6 cells). These differences became less expressed if they were related to the cell volume (Dragúň *et al.*, 1983) representing 30—50 per cent of the extracellular concentration from DHPA.

The present data show that in the variation of sensitivity of the same virus to antiviral substance in different cell type cultures, both the transport and metabolic conversion of the compound are involved. However, it seems very probable that some other factors (namely the enzymes both either anabolizing or catabolizing nucleosides and nucleotides) may participate as well.

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