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METABOLISM OF THE BROAD-SPECTRUM ANTIVIRAL AGENT, 9-(S)-(2,3-DIHYDROXYPROPYL) ADENINE, IN DIFFERENT CELL LINES

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Summary. — 9-(S)-(2,3-dihydroxypropyl)adenine [(S)-DHPA] did not undergo metabolic changes in any of the eleven cell lines tested. In these, it exhibited a varying antiviral activity against vesicular stomatitis virus (VSV), which appeared to correlate with the relative amounts of (S)-DHPA that had become cell-associated during virus infection.

Key words: *9-(S)-(2,3-dihydroxypropyl)adenine*; *antiviral effect*; *vesicular stomatitis virus*

Introduction

9-(S)-(2,3-dihydroxypropyl)adenine [(S)-DHPA] exhibits a broad-spectrum antiviral activity, encompassing both RNA and DNA viruses (De Clercq *et al.*, 1978). Its activity is most prominent against rhabdoviruses (namely VSV and rabies virus) (Sodja and Holý, 1980). In mice, (S)-DHPA is rapidly excreted, mostly unchanged from the body; the only catabolites of (S)-DHPA so far identified have been the 9-(S)-2,3-dihydroxypropyl derivatives of hypoxanthine, xanthine and uric acid (Čihák and Holý, 1978; Holý and Čihák, 1981). (S)-DHPA has no acute or subacute toxicity for mice, except for a reversible inhibition of spermatogenesis upon oral administration of high drug doses (De Clercq *et al.*, 1981). (S)-DHPA is a powerful inhibitor of S-adenosyl-L-homocysteine hydrolase; it has been suggested that this effect might be one of the reasons for its antiviral activity (Votruba and Holý, 1980). The antiviral action of DHPA varies considerably from one cell line to another (De Clercq *et al.*, 1978). Thus, it seemed of interest to investigate the dependence of the antiviral activity of (S)-DHPA upon the cell line used and to analyse the cell content with regard to the intracellular level of the drug and its eventual metabolites.

Materials and Methods

The following cell lines were used; PRK (primary rabbit kidney), Vero (a simian fibroblast cell line derived from African green monkey kidney). HeLa (a human epithelial cell line derived from a cervical carcinoma), RK 13 (a continuous cell line of human kidney), BSC-1 (a simian

epithelial cell line derived from African green monkey kidney), FL (feline lung fibroblasts) HEp-2 (a human epithelial cell line derived from a larynx carcinoma), ESM (human embryonic skin-muscle fibroblasts), T-21 (human fibroblasts trisomic for chromosome 21) and VGS (human diploid fibroblasts).

The confluent cell monolayers in 60 mm Falcon Petri dishes (approx. 10^6 cells per Petri dish) were inoculated with 100 $CCID_{50}$ (= 50% cell culture infectious dose, i.e. with approx. 50 $TCID_{50}$ per cell) of VSV. After 1 hr incubation at 37 °C, the residual virus was removed, cell cultures were washed with Eagle's minimal essential medium (MEM) and incubated with MEM (1 ml) containing 100 μg (S)-DHPA and 370 kBq (U-^{14}C-adenine)-(S)-DHPA (specific radioactivity 10.36 GBq/ /mmol; purchased from the Institute for Research, Production and Application of Radioisotopes, Prague, Czechoslovakia; radiochemical purity 99.9%) per Petri dish. Mock-infected cell cultures were run in parallel. After 24 hr incubation at 37 °C, the cell cultures were washed four times with cold Dulbecco's phosphate buffered saline (PBS) and frozen at −20 °C. After thawing, the cells were scraped off from the Petri dishes into 1 ml bidistilled water and disrupted by treatment for 20 sec at 0°C in a 100 W ultrasonic disintegrator (MSE) at 20 kHz at maximum output (8 μm peak-to-peak). To all samples 0.1 ml 50% aqueous trichloracetic acid (TCA) was added and after 20 min incubation at 0°C, the mixtures were filtered through Gelman type A/E glass fiber filters (Gelman Instrument Co., Ann Arbor, Michigan, U.S.A.) and washed with 5% TCA solution. The filtrates were shaken vigorously for 2 min with an equal volume of dry ether layer removed and the procedure repeated 5 times until the pH of the aqueous phase attained 4.5—5.0. The aliquots of the aqueous solution were used for the estimation of total radioactivity and the bulk dried on 2 cm strips of Whatman No 3 MM paper, with 1 OD_{260} DHPA as a carrier and AMP, ADP, Ino and DHPx [9-(S)-(2,3-dihydroxypropyl)hypoxanthin] as standards.

After development in 2-propanol — aqueous ammonia — water (7 : 1 : 2, v/v) overnight, the paper strips were dried and scanned in a Packard Model 385 apparatus. The radioactivity contents of the individual peaks were estimated by integration of the peak areas.

The individual radioactivity peaks on paper strips were soluted by water (1 ml) and the eluates were freeze-dried. The radioactive components were identified as follows: (a) AMP and ADP (R_F; 0.05 and 0.02, respectively) were incubated with 0.48 units of bacterial alkaline phosphatase (Worthington, U.S.A.) in 0.20 ml of 0.02 mol/l Tris-HCl buffer (pH 8.2) at 37 °C for 3 hr. The 30 μl-portions were spotted on separate sheets of Whatman No 3 MM paper with DHPA, Ado and dAdo as standards. Paper chromatography and electrophoresis in systems 1—4 (Table 2) followed by scanning revealed a single radioactivity spot at the position of adenosine. (b) Inosine (R_F; 0.38) was characterized by paper chromatography and electrophoresis in systems 1—4 (Table 2); scanning with Ino, DHPx, DHPA, Ado, Ade and Hx as standards revealed inosine as the only radioactive material in the eluate; (c) (S)-DHPA (R_F; 0.52) was identified similarly as inosine in systems 1—4 with DHPA, Ado, Ino, Hx and Ade as standards.

Results and Discussion

The cell associated [^{14}C]DHPA contents are presented in Table 1. It shows that the only radioactive components found in the cell homogenates consisted invariably of (S)-DHPA and adenine metabolites. (S)-DHPA did not undergo metabolic changes in any cell line tested. Particularly, the presence of any phosphorylated forms (DHPA mono-, di- or triphosphate) or its deaminated derivative (DHPAa) could be excluded. In addition to (S)-DHPA, the cell extracts contained small amounts of AMP and in some cases ADP and Ino. The identity of the adenosine nucleotides AMP and ADP was unequivocally proved by enzymatic dephosphorylation which resulted in the formation of adenosine, as indicated by chromatography and borate electrophoresis (Table 2). Adenine was not found in any of the cell extracts. Since the hypothetical conversion of (S)-DHPA to adenosine nucleotides would have to proceed via adenine formation and since the summation of these metabolites did not exceed the actual radiochemical impurity (^{14}C)adenine in the

Table 1. Cell associated radioactivity of ^{14}C-DHPA and antiviral activity of (S)-DHPA in different cellines

Cell line	Treatment	(^{24}C)DHPA content*	V/C	ID_{50}**
PRK	C	0.38	1.30	48
	V	0.49		
Vero	C	0.45	0.44	600
	V	0.20		
HeLa	C	0.92	1.10	190
	V	1.01		
RK 13	C	1.09	1.25	34
	V	1.37		
HK	C	0.53	1.33	190
	V	0.71		
BS-C-1	C	2.14	0.35	480
	V	0.74		
FL	C	0.44	1.45	34
	V	0.64		
HEp-2	C	2.29	1.09	48
	V	2.48		
ESM	C	1.21	0.91	145
	V	1.10		
T-21	C	1.28	1.03	48
	V	1.32		
VGS	C	2.65	1.15	48
	V	3.05		

C = control cells, V = VSV-infected cells, V/C = ratio of (^{14}C) DHPA content in cells.
* Expressed in % of total radicactivity input per 10^6 cells.
** 50% inhibitory dose of (S)-DHPA in μmol/l required to inhibit the cytopathic effect of VSV by 50%.

(^{14}C)DHPA preparation, it is obvious that these metabolites originated from the (^{14}C)adenine contaminant and not from (^{14}C)DHPA itself.

The actual concentration of DHPA in the cell homogenates amounted to 0.3—3.0% of the total input per 10^6 cells. The concentration of DHPA per cell volume amounted to approximately 1 mmol/l. This value is comparable with those of the natural adenine metabolites (e.g. AMP 0.4 mmol/l; ADP, 1 mmol/l) (Ueland and Saebø, 1979).

Among the various cell lines tested no significant differences were observed in the absolute amounts of (^{14}C)DHPA that were taken up by either virus- or mock-infected cells (Table 1). However, a direct correlation was noted between the relative content of the drug in the VSV-infected as compared to control cells (V/C) and the antiviral activity of (S)-DHPA in these cells (ID_{50}). The relationship between V/C and log ID_{50} was assessed by linear regression analysis. The correlation coefficient (r) was —0.806. A significant antiviral effect was only obtained for those cell lines that showed a V/C ratio higher than 1. Thus, the antiviral activity of (S)-DHPA may be related to an increased intracellular concentration of the drug following virus infection. This increased cell-association of (S)-DHPA might be due to an

Table 2. Chromatographic and electrophoretic constants for (S)-DHPA, adenine and their metabolites

Compound	Chromatography* R_F		Electrophoresis** E_{AMP}	
	(1)	(2)	(3)	(4)
DHPA	0.52	0.29	−0.14	0.04
DHPx	0.32	0.16	0.04	0.32
DHPx	0.26	—	0.54	0.71
Ado	0.57	0.35	−0.12	0.38
Ino	0.38	0.11	0.04	0.70
XaO	0.25	—	0.46	0.97
Ade	0.54	0.50	−0.10	0.05
Hx	0.36	—	0.08	0.17
dAdo	0.65	—	0.10	0.12
AMP	0.05	0	+1.00	+1.00
ADP	0.02	0	—	—

* Paper chromatography in (1) 2-propanol — ammonia — water (7 : 1 : 2) or in (2) 1-butanol — — acetic acid — water.

** Paper electrophoresis (with AMP as reference) (20 V/cm) on Whatman No 3 MM paper in (3) 0.1 mol/l triethylammonium hydrogen carbonate (pH 7.5) or in (4) 0.1 mol/l triethylammonium borate (pH 7.5).

increased uptake, decreased efflux or complex formation of the drug with some viral or cellular proteins that are specifically induced upon virus infection.

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