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EFFECT OF 9-(S)-(2,3-DIHYDROXYPROPYL)ADENINE ON EXPERIMENTAL RABIES INFECTION IN LABORATORY MICE

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Summary. — 9-(S)-(2,3-dihydroxypropyl)adenine (DHPA) exerted a pronounced influence on experimental rabies infection in laboratory mice. It was effective in a dose range from 1 to 100 mg/kg body weight. Intracerebral infection tended to be potentiated, intramuscular infection was partly or fully inhibited by the drug. Peroral administration was the most effective. The results of individual experiments varied and depended on the sensitivity of the virus strains employed, the dose of DHPA given and the time pattern of its administration.

Key words: *rabies*; *antiviral nucleoside analogue*; *mice*

Introduction

A novel nucleoside analogue, 9-(S)-(2,3-dihydroxypropyl-adenine (DHPA; Fig. 1) was recently reported to possess potent antiviral activity (DeClercq *et al.*, 1978; DeClercq and Holý, 1979) against some DNA and RNA viruses.

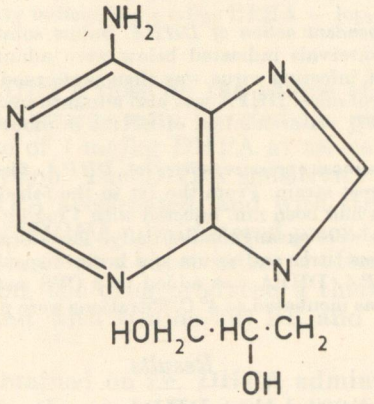

Fig. 1.
9-(S)-(2,3-dihydroxypropyl)adenine

Though it does not exhibit general activity within the two virus groups (it was active against vaccinia and herpes simplex type 1 and 2 viruses of the DNA viruses and against vesicular stomatitis and measles viruses of the RNA viruses but had no effect on polio, coxsackie and Sindbis viruses), its extremely low cytotoxicity and acute toxicity combine to give a very favourable chemotherapeutic index. The drug is not significantly metabolized in vivo in mice (Čihák and Holý, 1978; Holý and Čihák, 1980) and is rapidly excreted from the organism. Though the mechanism of its antiviral action is not yet known with certainty, experiments on Rous sarcoma virus have shown that the drug acts upon protein kinases (Kára *et al.*, 1979) and in vitro experiments which demonstrated an inhibitory activity of the drug upon S-adenosyl-L-homocysteine hydrolase (Votruba and Holý, 1980) suggest that nucleic acid methylation processes might be the target of its action.

The impressive activity of DHPA against vesicular stomatitis virus, which is by far the most sensitive of the viruses tested (DeClercq *et al.*, 1978), stimulated us to investigate the action of this compound on rabies virus in mice in vivo.

Materials and Methods

DHPA was prepared by the procedure described by Holý (1975).

Rabies virus. Strains street (of fox origin). CVS and strains 297 BF, 66B and 133 SG isolated from small wild rodents (Sodja *et al.*, 1971) were used in the form of 10% mouse brain suspensions in saline. The lethal time for mice of all the strains was 7 ± 1 days.

Inoculation of mice was performed according to Koprowski (1973); LD_{50} was calculated by the method of Reed and Muench. Mice weighing 9—10 g each were inoculated intracerebrally (i.c.) or intramuscularly (i.m.) with 0.03 ml or 0.1 ml of virus, respectively.

Virus neutralization (VN) tests were performed by the method of Atanasiu (1973), 30—50 LD_{50} of strain CVS having been used in the reaction. Sera and 10% brain suspensions were inactivated at 56 °C for 30 min.

DHPA toxicity test. Mice were injected i.c. or i.m. with 0.1 to 100 mg DHPA per kg body weight.

Quantitative and time-dependent action of DHPA. Saline solutions of DHPA in the weight amounts and at the time intervals indicated below were administered i.c. or i.m. to mouse groups in which titration of infecting virus was then performed. LD_{50} values were calculated at the time of death of control mice. DHPA was also administered perorally; in this case drinking water was replaced by a 0.1% water solution of DHPA in mouse cages at the terms indicated in Table 4.

Virus-inhibiting and immunosuppressive effects of DHPA. Groups of mice were inoculated i.m. with 36 LD_{50} of the street strain. From the 1st to the 5th day after infection, five control mice and five animals which had been i.m. injected with 1 mg/kg DHPA 2 hr before and 1, 2, 4 or 24 hr post infection (p.i.) were exsanguinated daily. Their brains and sera were pooled each separately and the brain virus titres and serum and brain-suspension VN titres were estimated.

Virus inactivation by DHPA. DHPA was added to a CVS suspension to a concentration of 10 mg/ml and the mixture was incubated at 4° C. Titrations were performed at intervals for 24 hr.

Results

Control tests had indicated that DHPA was not toxic for mice if administered i.c. or i.m. in doses ranging from 0.1 to 100 mg/kg, i.e. in doses found to be effective against the rabies virus strains employed.

Table 1. Effects of a single intramuscular dose of DHPA (1 mg/kg) on infection of white mice by rabies virus strains

Route of infection	Strain	Day p.i.	DHPA administered on day −2	−1	0	+1	+2
i.c.	297 BF	5	−0.26*	1.87	1.11	0.24	0.24
		6	0.75	2.17	2.66	0.95	0.74
		7	0.87	2.26	2.69	1.76	1.01
		8	0.61	2.24	2.33	2.16	0.75
	street	5	1.00	2.00	1.50	2.00	0.86
		6	0	0.75	0.77	0.75	0.86
		7	−0.92	−0.66	−0.42	−0.55	0.16
		8	−0.92	−0.66	0.08	−0.55	0.16
	CVS	5	0	0.16	0	0	0
		6	0.84	0.79	−0.16	−0.16	−0.16
		7	1.34	1.09	0.08	−0.16	−0.16
		8	1.68	1.95	0.08	−0.16	−0.16
i.m.	297 BF	5	−1.50	−1.50	−1.50	−0.75	−1.50
		6	−1.50	−1.50	−1.50	−0.75	−1.50
		7	−1.50	−1.50	−1.50	−0.75	−1.50
		8	−1.50	−1.50	−1.50	−0.75	−1.50
	street	5	−1.45	0.30	0.30	−1.45	−1.45
		6	−1.45	0.30	0.30	−1.45	−1.46
		7	−0.71	0.30	0.30	−1.45	−0.71
		8	−0.71	0.30	0.30	−1.45	−0.71
	CVS	5	−0.76	0	−1.50	−1.50	0
		6	0	0	−1.50	−1.50	0
		7	0	0	−1.50	−1.50	0
		8	0	0	−1.50	−1.50	0

* The values represent mortality indices ($\log_{10}$ LD_{50} DPHA − $\log_{10}$ LD_{50} control).

The effect of a single DHPA dose on the course of mouse infection is illustrated in Table 1. The strains were titrated i.c. and i.m. in mice which had received an i.m. dose of 1 mg/kg DHPA at terms between 2 days prior to and 2 days after infection. The DHPA sensitivity of the strains varied. In i.c. infected mice DHPA accelerated, and with strains 297 BF and CVS even enhanced, mortality (DHPA administered prior to infection with strain CVS; simultaneously with, or at a short interval after infection with strain 297 BF). On i.m. infection, the drug produced a therapeutic effect with all the virus strains used and with strains 297 BF and street also a prophylactic effect.

Similar results were obtained on i.c. DHPA administration (not shown). The effective dose was in the range from 0.1−100 mg/kg DHPA by both administration routes. Complete inhibition of virus multiplication (1.50 $\log_{10}$ LD_{50}) by DHPA was only observed on i.m. infection (see also below).

Table 2. Effects of single and repeated i.m. administration of DHPA on i.m. lethality of street rabies virus for white mice (in per cent LD_{50} of control mice)

Effect of number of doses (regardless of time of administration and total dose). Number of doses:	1	27.7 ± 3.0%
	2	43.5 ± 3.5%
	3	47.0 ± 2.0%
	4	29.0 ± 7.0%
Effect of DHPA administration starting time (regardless of total dose and No. of injections). Started on day p.i.:	0	36.0 ± 3.5%
	1	37.0 ± 5.0%
	2	36.4 ± 3.5%
Effect of infection stage; DHPA given only on day p.i.	0	36.0 ± 3.5%
	1	32.7 ± 3.5%
	2	33.8 ± 2.5%
	3	34.7 ± 3.0%
Effect of total dose (mg/kg):	0.5—20.0	34.6 ± 2.0%
	0.5— 1.0	33.2 ± 3.5%
	1.0—20.0	38.5 ± 3.0%

In another series of experiments the DHPA dose was administered in several portions. Prophylactic i.m. inoculation of 1 mg/kg DHPA produced the same effect on the course of i.c. and i.m. infection with street virus or strain 297 BF as fractionation of this dose into two 0.5 mg/kg or three 0.3 mg/kg portions given on 2 or 3 consecutive days prior to infection.

To test the therapeutic effect, mice infected i.m. with street virus recived the drug either as a single 1 or 10 mg/kg dose or subdivided into several doses each equalling 50, 33 or 25% of the basic weight amount. In both cases DHPA administration was started at the time of infection or up to three days later. The drug significantly reduced mortality, irrespective of whether it was administered simultaneously with, or two days after the virus. Dose fractionation by day was of little significance. It appeared that a single dose given during day 0 to 3 p.i. sufficed to reduce mortality. Nor was an essential difference in mortality reduction found after a total dose of 0.5 mg/kg or 20 mg/kg (Table 2).

Table 3. Therapeutic effect of repeated i.m. doses of 10 mg/kg DHPA given 2, 4, 6 and 8 hr after i.m. inoculation

Strain	$\log_{10} LD_{50}$		Mortality reduction
	Control	DHPA	
297 BF	2.25	1.74	69.2%
CVS	3.00	1.75	94.4%

Table 4. Effect of DHPA administered with drinking water (0.1% water solution) on the course of rabies infection

Strain	Route of infection	Virus titres (log_{10} LD_{50})				
		DHPA administered on day p.i.				
		Control	−1	0	1	2
133 SG	i.c.	3.25	2.00	3.57	4.56	3.00
	i.m.	1.55	0	0	0	0
66 B	i.c.	6.74	6.50	6.74	5.43	7.50
	i.m.	2.25	0	0	2.50	2.50

The administration of DHPA in a single dose or at daily intervals significantly lowered but seldom eliminated the mortality of infected animals. In view of the infectious cycle of the virus strains tested and DHPA half-life (2 hr in the mouse organism after intraperitoneal administration), the therapeutic effect of the drug was assayed after repeated i.m. administration of 10 mg/kg amounts at 2, 4, 6 and 8 hr p.i. (Table 3). Complete prevention of mortality was not achieved with the 297 BF strain even in this way. But the same weight amounts administered i.m. at 2, 4, 8 or 24 hr after i.m. inoculation of 3 LD_{50} of CVS eliminated mortality (not shown in Table 3). DHPA given 48 hr p.i. no longer had any protective effect.

As an alternative approach, DHPA was administered perorally in the form of a 0.1% water solution given as drinking water. The results (Table 4) showed that, although the drug again practically had no effect against i.c. infection, it completely prevented mortality in mice infected i.m. with strain 133 SG and, if administered within 24 hr p.i., also with strain 66 B.

DHPA had no direct inactivating effect on rabies virus. If incubated with the virus at 4° C, it did not substantially influence its titre even after 24 hr. However, the drug affected propagation of the virus, as was demonstrated

Table 5. Virus-inhibiting and immunosuppressive effect of DHPA on i.m. infection of mice by street virus

DHPA given i.m. at hr p.i.	3 days p.i. VN titre (log_{10})		Virus (log_{10} LD_{50})	5 days p.i. VN titre (log_{10})	
	Serum	Brain		Serum	Brain
Control	2.20	1.54	4.74	2.01	2.20
−2	1.06	0	0	1.90	0
0	1.59	0.86	4.54	1.90	0
1	0	0	0	1.54	0
2	1.30	0	4.50	1.06	0
4	1.30	0	0	1.30	1.00
24	2.20	1.59	0	2.20	0

in an experiment in which groups of mice were i.m. infected with 36 LD_{50} of street virus and given i.m. 1 mg/kg DHPA at the intervals indicated in Table 5. Up to and inducing day(3, virus was not detectable in the brains of these and control animals, although their sera and brain suspensions displayed a low VN activity. An antiviral and immunosuppressive effect was demonstrated on day 5 p.i. (The infectious titres found after DHPA administration simultaneously with the virus and 2 hr p.i. were probably due to virus from the brains of mice in which for some reason virus multiplication had not been inhibited and which were added into the pool.)

Discussion

A street rabies virus strain, a laboratory strain and several mouse strains, which resemble street strains by some of their properties and laboratory strains by others (Sodja and Matouch, 1972), were used in the present experiments. Pilot tests on the possibility of using DHPA in experimental rabies therapy were very promising. But further investigations revealed a variability of DHPA anti viral activity with respect to strain sensitivity and the optimum timing, dosage and route of virus inoculation and of DHPA administration. A similar variability in DHPA effect was found among herpes simplex virus type 1 strains (DeClercq *et al.*, 1978). Multiplication variability in vivo among different rabies virus strains is known (Murphy, 1977).

A potentiation of virus lethality by DHPA was especially encountered on i.c. infection, i.e. a form the occurrence of which is precluded in nature. The maximum increase in lethality by 2.69 $\log_{10}$ units was caused by the drug administered i.c. 2 days prior to i.c. virus inoculation. The highest reductions in i.c. LD_{50} values were 1.39 and 1.15 $\log_{10}$ units after i.m. and i.c. administration of DHPA, respectively, Čihák and Holý (1978) demonstrated that on intraperitoneal administration only about 0.04% of the DHPA applied penetrates into the mouse brain in 2 hr.

But the lethality of i.m. injected virus was reduced by at least 90% in 39 out of 80 experiments in which DHPA was administered i.m., disregarding the size and number of drug doses given. After i.c. injection of DHPA, lethality was reduced 28 out of 60 times and left unaffected 29 times.

It seems that a dose of 1 mg/kg/day, despite having reduced mouse lethality by over 90% in some instances, was too low as a therapeutic dose, as was unequivocally confirmed by the results obtained after short-interval repetition of a 10 mg/kg dose over the several first hr p.i. A greater antiviral effect was achieved by repeating DHPA administration at shorter intervals rather than by increasing its total weight amount. One should also bear in mind that in most experiments a higher infecting virus dose was used than is supposedly transmitted in natural infection.

To act in a virus-inhibiting manner, DHPA had to be administered early and its level had to be appropriately maintained in the organism (fraction-

ated doses at short intervals or administration via drinking water). The therapeutic effect of DHPA under simultaneous active or passive immunization was not investigated. Furthermore, one cannot preclude the possibility of an advantageous synergism in antiviral action between DHPA and adenine arabinoside (DeClercq *et al.*, 1978) or 6-azauridine (Rada and Holý, 1980), as has been described for vesicular stomatitis and vaccinia viruses.

The experiments did not provide sufficiently clear evidence whether the elimination of VN activity from the central nervous system results from as immunosuppressive action of DHPA on white blood cells or from a depression of the virus inhibited by DHPA.

In view of the growth specificity of rabies virus in tissue cultures (Wiktor, 1973), it would probably be impossible, to study in detail the mechanism of antirabies action of DHPA in vitro with the use of defined clones. The present results suggest that there are two possible mechanisms of DHPA action on rabies virus: (*a*) a "direct" effect, perhaps by virus maturation being affected (effectivness of doses repeated at short intervals and peroral administration), and (*b*) a "mediated" effect, via an unknown pathway, as suggested by the antiviral activity at a time when the substance can no longer be present in the macroorganism (prophylactic effect of small doses given at daily intervals).

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