

STUDIES ON THE EFFECT
OF 9-(R, S)-(2,3-DIHYDROXYPROPYL)ADENINE
ON TICK-BORNE ENCEPHALITIS VIRUS

M. GREŠÍKOVÁ, B. RADA, A. HOLÝ*

Institute of Virology, Slovak Academy of Sciences, 817 03 Bratislava
and *Institute of Organic Chemistry and Biochemistry,
Czechoslovak Academy of Sciences, 166 10 Praha 6, Czechoslovakia

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Summary. — Peroral treatment with 9-(R, S)-(2,3-dihydroxypropyl) adenine did not protect mice infected with tick-borne encephalitis (TBE) viruses (strains Hypr and Ir13) when the drug was administered at the time of infection and/or up to day 2 after infection. The analogue inhibited the haemagglutination activity of the Skalica strain *in vitro*.

Key words: 9-(R, S)-(2,3-dihydroxypropyl) adenine; *in vivo* inhibition of virus replication; tick-borne encephalitis virus

9-(R, S)-(2,3-dihydroxypropyl)adenine [(R, S)-DHPA], a nucleoside analogue with aliphatic chain resembling a portion of ribose moiety, was shown to inhibit replication of several RNA and DNA viruses (De Clercq *et al.*, 1978, Rada and Holý, 1980; Sodja and Holý, 1980). In the present experiments we report that this analogue has not shown any protective effect in mice infected with tick-borne encephalitis virus (TBE) strains when applied at the time of infection and/or up to day 2 after infection.

(S)-enantiomer of DHPA is the active substance in inhibition of virus replication (De Clercq and Holý, 1979); in these experiments the racemic form of (R, C)-DHPA was used, since practical factors such as ease and the cost of preparation gave it some advantage over (S)-enantiomer. A double dose of that required with (S)-enantiomer (Sojda and Holý, 1980) was administered throughout. (R, S)-DHPA was prepared as described by Holý (1975).

White mice from the bread Velaz (Czechoslovak radom bread) weighing 10-12 g were infected subcutaneously with $10-10^7$ LD₅₀ of Hypr and Ir13 strains of TBE, respectively. Hypr strain is the prototype strain of TBE virus, Ir13 strain has been isolated from *Ixodes ricinus* ticks in 1978 (Grešíková and Sekeyová, 1980). Mice received (R, S)-DHPA *ad libidum* in drinking water (0.2% solution).

Table 1 shows that the substance did not affect the mortality of mice when given at the time of infection and/or up to 2 days after infection. Some inhibitory effect was observed in one experiment only, when the substance was administered 24 hr before virus infection. In our previous experiments with several arboviruses (Grešíková and Rada, 1972), the inhibition of haemagglutination was detected by another analogue of 6-azauridine. Under conditions of optimal pH, the effect of (R, S)-DHPA on haemagglutinin of four TBE viruses was studied. Viral haemagglutinins were prepared by sucrose-acetone

Table 1. Effect of DHPA administered in drinking water on mice infected with TBE virus

Experiment No. - Virus strain	Control	Days of DHPA administration			
		-1	0	1	2
I Hypr	8.5*	7.7	8.5	8.5	8.5
II Hypr	9.5	9.5	9.5	9.5	9.5
I Ir13	8.5	7.5	8.5	8.5	8.5
II Ir13	9.5	9.5	9.5	9.5	9.5

* Titres expressed in subcutaneous log LD₅₀/0.01 ml.

The same virus strain was inoculated in different dilutions to mice by subcutaneous route; the end point titres in the presence and absence of the drug were determined.

Day 0 = therapy since the day of virus administration.

extraction and exposed to a maximal nontoxic concentration (3 mg/ml) of (R, S)-DHPA for 18 hr at 4 °C. As shown in Table 2, an evident direct inhibitory effect of DHPA on haemagglutinin of the Skalica strain was observed. Infected mouse brain suspension containing the Hypr virus was exposed to (R, S)-DHPA (3 mg/ml) for 18 hr at 4 °C; no difference in infectivity titre between the material incubated in the presence or absence of the analogue was found.

Table 2. Effect of DHPA on the haemagglutinins of certain TBE virus strains

Virus	pH*	Haemagglutination titres	
		Untreated	DHPA** 3 mg/ml
Hypr	6.4	2560	1280
Ir13	6.4	1280	640
Skalica	6.6	160	20
Powassan	6.6	160	80

* Optimal pH used in haemagglutination test.

** Haemagglutinin extracted by sucrose-acetone was treated with the drug.

The presented data showed that the inhibition of haemagglutination may differ in various tick-borne encephalitis virus strains. It is of interest that vesicular stomatitis and rabies viruses (enveloped — RNA viruses from family Rhabdoviridae) were found sensitive to the analogue (De Clercq *et al.*, 1978; Sodja and Holý, 1980), while strains of TBE virus (enveloped + RNA viruses from family *Togaviridae*) were not inhibited.

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