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ANTIVIRAL ACTION OF DIPYRIDAMOLE AND ITS DERIVATIVES AGAINST INFLUENZA VIRUS A

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Summary. — Dipyridamole proved to be active against influenza viruses A/England 42/72, A/Bangkok 1/79 and A/fowl plague (FPV). The antiviral activities assayed by various methods varied from 90-99 per cent. No inhibition was found against influenza virus B/Leningrad 235/74 in vitro. Three dipyridamole derivatives were significantly activé in tissue cultures against influenza virus A/England 42/72 and A/FPV. In white mice infected with influenza virus A/England 42/72 dipyridamole administered orally showed a protection rate of 62.5 per cent.

Key words: *Influenza virus*; *dipyridamole*; *antiviral activity*

Introduction

In previous papers we reported about the inhibitory action of dipyridamole and its derivatives against numerous non-enveloped and enveloped RNA and DNA viruses as well as complete suppression of viral RNA synthesis of Mengo virus (Tonew *et al.*, 1977; Tonew and Dzeguze, 1977; Tonew and Fahlbusch, 1977; Tonew and Löber, 1978; Tonew *et al.*, 1978, 1979; Tonew, 1980). The action against other viruses in vitro was confirmed by Oehring and Schmidt (1978) and Bankowski *et al.* (1980), and in clinical trials by Günther *et al.* (1977).

Materials and Methods

Viruses and cell cultures. Fowl plague virus (FPV), strain Weybridge (Hav1Neq1), and influenza viruses A/England 42/72 (H3N2), A/Bangkok 1/79 (H3N2), and B/Leningrad 235/74 were passaged in chick embryos. Chick embryo cells (CEC) were trypsinized and prepared as described by Tonew and Tonew (1969). Madin Darby canine kidney cells (MDCK); for media and cultivation see Gaush and Smith (1968). Chorioallantoic membrane (CAM) cultures were prepared according to Horváth (1954).

Plaque assay and one-step growth cycle experiments were carried out as described by Tonew and Tonew (1971). For the plaque assay, influenzavirus-infected MDCK cells were overlaid with medium containing 10 μg/ml trypsin and 0.6 per cent agar Noble (Difco) into the overlay medium; for the assay of the cytopathic effect (CPE), 5 per cent trypsin in the maintenance medium was employed. The haemagglutinin was titrated according to Takatzuki and Tamura (1971).

Table 1. Action of dipyridamole on influenza virus strains in CAM cultures

Virus strains	Doses μmol/l	Decrease in virus titre (log_{10} ID_{50})
A/England 42/72	80	0.3
A/England 42/72	120	2.2
A/Bangkok 1/79	120	1.7
B/Leningrad 235/74	120	0.5

Mean values of 3 experiments.

Experiments in mice. White mice of an undefined strain sensitive to epidemic influenza virus strains, weighing 18—20 g, were intranasally infected with 10 LD_{50} in 0.05 ml of influenza virus A/England. The drug was administered by the oral route 2 hr before and 2, 24, 48 and 72 hr after infection or two times daily for 4 days. The statistical evaluation was performed by the method of Veckenstedt and Horn (1976).

Compounds used. Dipyridamole (AWD 565): 2,6-bis(dimethylamino)-4,8-dipiperidino-pyrimido-(5,4-d)pyrimidine, mol. wt. = 504.62; AWD 568: 2,4,8-triopiperidino-6-diethanolamino-pyrimido-(5,4-d) pyrimidine, mol. wt. = 484.63; AWD 628: 2,6-dipiperidino-4,8-bis-(N-methyl-N-ethanol-amino-pyrimido(5,4-d) pyrimidine, mol. wt: 444.6; AWD 642: 2.6-bis(N-methyl-N-ethanolamino)--4,8-bis n-propanolamino)-pyrimido(5,4-d)pyrimidine, mol. wt. = 424.5. The substances were kindly provided by Dr. H. Goldner, VEB Arzneimittelwerk, Dresden.

Results

Dipyridamole exerted a significant antiviral activity against influenza viruses A/England 42/72 and A/Bangkok 1/79 in the CAM test, with 99 and 98 per cent inhibition, respectively (Table 1). No significant activity was found against influenza virus B/Leningrad. Plaque reduction caused by 50 μmol/l dipyridamole amounted to 94 and 90 per cent, respectively, in CEC and MDCK cells (Fig. 1). A good protective effect against FPV was observed with dipyridamole derivatives AWD 566, 568 and 642: the inhibition

Table 2. Action of dipyridamole derivatives on the replication of influenza virus A/England 42/72 and FPV in CAM or CEC cultures

Compound	Virus strains	Dose* μmol/l	Decrease in virus titre (log_{10})	Per cent inhibition
AWD 566	A/England 42/72	46	0.5 ID_{50}	n.s.
	A/FPV	138	4.0 $TCID_{50}$	99.99
AWD 568	A/England 42/72	31	0.5 ID_{50}	n.s.
	A/FPV	206	5.0 $TCID_{50}$	> 99.99
AWD 628	A/England 42/72	22	0.7 ID_{50}	n.s.
AWD 642	A/England 42/72	94	2.0 ID_{50}	99

Mean values of 3 experiments.
n.s. = not significant; *maximal tolerated dose
Influenzavirus A/England was assayed in CAM cultures. Titre in untreated control: 5.5 log ID_{50}.
Influenzavirus A/FPV was assayed in CEC cultures; titre in untreated control: 7.83 log $TCID_{50}$/ /0.2 ml.

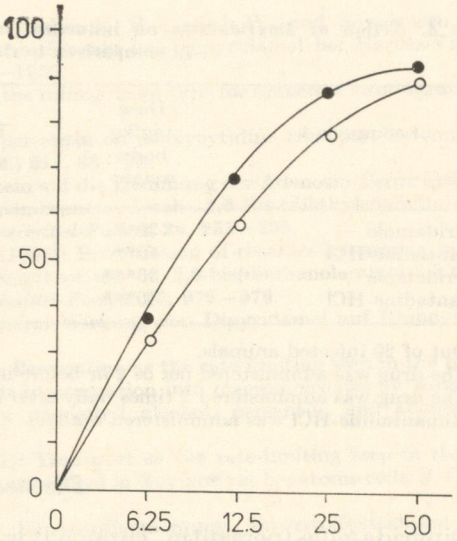

Fig. 1.
Effect of dipyridamole on influenza virus reproduction

CEC andMDCK cell monolayers in Dementer flaks were infected with FPV (●) and influenzavirus A/England (○). After 1 hr adsorption at room temperature, the virus was sucked off from the cell cultures and replaced with 6 ml overlay medium as described in Materials and Methods. Three flaks were used for each compound concentration. The titre reduction was calculated in per cent of the average plaque count of the virus control. Abscissa: concentration of dipyridamole in μmol/l; ordinate: plaque reduction in per cent of control

of infectious virus yield revealed in one-step growth cycle experiments in FPV-infected CEC reached approximately 99 per cent (Table 2). The reduction of haemagglutinin titre in the culture fluid was in accordance with these findings (Table 3). With the exception of AWD 642, the other derivatives showed no significant effect against influenza virus A/England 42/72.

In mice infected with virulent influenza virus A/England 42/72, dipyridamole showed a 62.5 per cent protection rate (Table 4).

Table 3. Haemagglutinin titres after treatment with dipyridamole of virus-infected MDCK and CEC cultures

Cells	Virus strains	Dipyridamole concentration μmol/l	Haemagglutinin titre
MDCK	A/England 42/72	50	—
		25	—
		12.5	—
		6.25	—
		0	40
CEC	A/FPV	50	—
		25	—
		12.5	—
		6.25	10
		0	160

Cells were infected at an input multiplicity of 20 PFU/cell and treated with the designated dipyridamole concentrations. After 80 hr of incubation at 37° C, the cells were threefold frozen and thawed, the supernatant was collected and assayed for haemagglutinins with 1 per cent chicken erythrocytes. — : result negative.

Table 4. Action of dipyridamole on influenza virus A/England 42/72 infection in white mice in comparison to rimantadine-HCl

of compound	Dose mg/kg body weight	No. of survivors* Treated	Control	Protection rate (%)
Dipyridamole	30**	14	4	62.5
Rimantadine-HCl	40**	18	4	87.5
Dipyridamole	30***	14	4	62.5
Rimantadine-HCl	40***	16	4	75

* Out of 20 infected animals.
**The drug was administered per os 2 hr before infection and 2, 24, 48 and 72 hr after infection.
***The drug was administered 2 times daily after infection for 4 days.
Rimantadine-HCl was administered orally.

Discussion

Dipyridamole (persantin, curantyl) is a well known coronary vasodilatator and antithrombotic drug as well as an excellent inhibitor of nucleoside transport into cells (Scholtissek, 1968; Kessel and Hall, 1970; Kopitar, 1970; Plagemann and Roth, 1969; Plagemann and Shea, 1971).

The present data demonstrated a significant antiviral activity of the drug in vitro against the influenzaviruses A/England 42/72, A/Bangkok 1/79 and A/FPV, and in vivo in mice against influenzavirus A/England 42/72.

The plaque reduction and the one-step growth cycle experiments showed a depression of plaque formation and infectious virus production of 90-99 per cent as compared to controls. The derivatives AWD 566, 568 and 642 significantly inhibited the reproduction of influenzavirus A/England 42/72 in the CAM test.

The maximum inhibitory concentration of dipyridamole is tolerated very well on oral administration. The timing of dipyridamole therapy against a virulent strain of influenza virus is quite important. We have seen a good efficacy when therapy was started with short time pretreatment by the drug before virus inoculation. The effect of two daily oral doses of the drug after virus infection for 4 days was not markedly different from that of pretreatment. Moreover, dipyridamole potentiates the reactive hyperaemia of coronary and peripheral blood circulation, thus increasing the oxygen consumption (Miura *et al.*, 1967; Schollmeyer and Jeschke, 1969), and in consequence improves the common resistance.

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