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Antiviral Activity of Dipyridamole in Experimental Viral Infections in Mice

Margarita Mastikova[1], Angel S. Galabov[2*], Alexander A. Karparov[1], Paulina Doseva-Runevska[1]

[1] *Queen Joanna Hospital/ISUL, Sofia*
[2] *The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria*

Abstract

Single application of the interferon inducer dipyridamole administered intraperitoneally or *per os* demonstrated a marked antiviral effect in mice with experimentally induced alphavirus infection –Semliki Forest (SFV) virus-caused encephalitis, applied orally in influenza virus A/Puerto Rico/8/34 (H1N1) and B/Lee/40 – respiratory infections, and herpes simplex virus type 1, strains Lennett and Leningrad 2 – encephalitis, and HSV-1 strain Leningrad 2-caused skin infection. In brain and respiratory infections the antiviral activity was manifested *via* the protection index, a decrease in the cumulative mortality percentage, and by the prolongation of the mean survival time. In the HSV-1 dermatitis suppression of symptomatology was observed.

Keywords: dipyridamole, interferon inducer, mice, SFV, HSV-1, influenza viruses A and B

Резюме

Еднократно приложение на индуктора на интерферон дипиридамол демонстрира отчетлив антивирусен ефект въвеждан интраперитонеално или пер ос в мишки с експериментално индуцирана алфавирусна инфекция– енцефалит след инокулиране на Semliki Forest virus, прилаган пер ос у мишки с респираторна инфекция с причинители грипни вируси A/Puerto Rico/8/34 (H1N1) и B/Lee/40, прилаган интраперитонеално или орално в мишки с енцефалит, индуциран с щамове Lennett или Ленинград 2 на вирус херпес симплекс тип 1, както и при кожна херпесна инфекция в мишки с щам Ленинград 2. При експериманталните енцефалити и респираторните инфекции антивирусната активност бе ргистрирана чрез индекса на протекция – намаление на процента на кумулативния леталитет и арез удължаване на средното време на преживяване. При херепсния дерматит бе отчетено подтискане развитието на симптоматиката.

Introduction

Tonew and Dzeguze (1977), M. Tonew *et al*. (1977) and E. Tonew *et al*. (1982) reported about the antiviral activity of pyrimido[5,4-d]-pyrimidine (dipyridamole, DP) when tested in vitro against a wide range of RNA- and DNA- containing viruses. These findings were confirmed and extended by *in vitro* studies on the antiviral effect of this compound carried out by Oehring and Schmidt (1978), Bankowski *et al*. (1981), Korbecki *et al*. (1985), partially by Snoek *et al*. (1994).

A new direction on the study of DP as an antiviral substance was traced after our communications (Galabov and Mastikova, 1980, 1982; Galabov *et al*., 1985) that this substance is an interferon (IFN) inducer. We suggested that the antiviral activity of DP could be entirely due to its IFN-inducing capacity. This effect was particularly well expressed in experiments *in vivo* involving oral administration of the compound to mice, and in investigations in human volunteers (Galabov and Mastikoiva, 1982, 1983a,b; Galabov *et al*., 1987; Grigoryan *et al*., 1989; Konstantinov *et al*., 1989).

* Corresponding author: galabov@microbio.bas.bg

Our further studies were directed towards *in vivo* testing the efficacy of DP as an antiviral agent when administered as an IFN inducer, namely, (a) prophylactic single-dose administration (e.g. 24 h prior to virus inoculation or in the latency period of the infectiion), and (b) administration of DP at IFN-inducing doses.

The antiviral effect of DP was tested in four different models of experimental viral infections in mice infected with: (a) Semliki Forest virus (SFV) causing encephalitis; (b) influenza viruses A/Puerto Rico/8/34 (H1N1) and B/Lee; (c) herpes simplex virus (HSV) type 1, strains Lennet and Leningrad 2 encephalitis; (d) HSV type 1, Leningrad 2 strain skin infection.

Materials and Methods

Compound

2,6-(Dietanolamino)-4,8-dipiperidino-pyrimido-(5,4-d)pyrimidine (dipyridamole, DP). For *in vivo* experiments Antistenocardin 2 ml ampouls, 0.5% solution (Pharmachim, Sofia) was used. The working dilutions were prepared in a solvent consisting of tartaric acid 0.2%, polyethylene glycole 600 5%, distilled water to 100%. This solvent was used as placebo.

Test animals

Random-bred line H or Swiss albino mice, as well as inbred line C-57/Black mice.

Viruses

Semliki Forest virus (SFV) was cultivated via passages in primary cell culture of chick embryo cells and 2-3 intracerebral passages in newborn albino mice. Working virus represented 10% brain suspension.Influenza viruses A/Puerto Rico/8/34 (H1N1) and B/Lee/40 were subjected to serial intranasal passages in albino mice. The stock virus represented lung suspension with an infectious titre of $10^{4.3}$ $LD_{50/ml}$ and $10^{5.5}$ $LD_{50/ml}$, respectively. Virus herpes simplex (HSV) type 1, strains Lennett and Leningrad 2, underwent serial passages in human embryonal lung diploid fibroblasts.

Experimental infection with Semliki Forest virus in albino mice

Albino mice of the random-bred line H, weighing 10-12 g (15-20 animals per test group) were infected intraperitoneally (i. p.) with 10 or 100 MLD50 of SFV. DP was administered singly, i. p. or *per os* 24 hours before, 24 or 72 hours after virus inoculation. The observation period was 30 days. The cumulative mortality percentage, with evaluation of the protection coefficient (PC) and the protection index (PI), and the MST in the treated and the placebo groups were determined.

Experimental infections in mice with influenza viruses A and B

Albino mice with a body weight of 10-12 g of the rendombred line H (15 animals per test group) were infected intranasaly with influenza virus A/ Puerto Rico/8/34 (H1N1) or B/Lee/40 (under ether anesthesia). DP was administered singly orally 24 h prior to, 24 or 72 h post virus inoculation. The observation period was 15 days. The cumulative mortality percentage and MST in the treated and placebo groups was recorded.

Infection with HSV type 1 (experimental herpetic encephalitis) in albino mice

Albino mice (15-20 animals per test group) were infected i. p. with 10 or 100 MLD_{50} of HSV-1 strains Lennett or Leningrad 2. DP was administered singly i. p. or per os 24 hours before, 24 or 72 hours post virus inoculation. Observation period was 30 days. The cumulative mortality percentage and the MST in the tests and the placebo groups were checked.

Experimental HSV-1 induced skin infection

Mice C57/Black weighing 20 g (14 animals per test group) were infected via rubbing of undiluted tissue culture virus with a titre of $10^{6.33}TCID_{50}$ in the scarified preliminarily de-haired skin on the mouse back. DP was administered singly i. p. 24 hours before, 72 hours or 120 hours post virus inoculation. The chronology of the appearance and the development of the local herpetic lesions in the test and the placebo groups were recorded.

Statistical treatment and analysis of the experimental data

The testing of DP antiviral activity in vivo the protection coefficient (PC) and the protection index (PC), as well as the MST were evaluated.

PC = mortality % in the placebo group / mortality % in the DP treated group

PI = PC-1 / PC x 100

The statistical treatment of the mortality data included determination of (a) the mean error (m_p) according to the formula

$m_p = \pm\sqrt{\mathbf{p}(100-\mathbf{p}) / n-1}$,

where **p** is the mortality percentage,

n –number of animals per test group

and (b) by the values of t, evaluated according to Ilyenko (1977).

Results

Effect of DP in experimental alphavirus (SFV) infection

DP was administered i. p. 24 h before virus inoculation with single doses of 0.1, 1.8, 5.5 and 16.7 mg/kg, a dose range corresponding to the IFN-

inducing doses of this substance in albino mice. In Fig. 1A it is seen the effect of this compound during infection with 10 LD_{50} of SFV, corresponding to approximately LD_{95}. DP at a dose of 0.1 mg/kg reduces mortality to 66.7%. With higher doses, the effect was slightly increased, reaching a maximum at 5.5 and 16.7 mg/kg - 50% of the mice survived and the mean survival time (MST) was extended by 9.7 days.

The protective effect was more strongly pronounced in infection with 100 LD_{50} (Fig. 1B). When DP was administered in doses of 5.5 and 16.7 mg/kg the protection rates were 46.7% an 50%, respectively, and a prolongation to 19.5 days in the MST was observed, while the MST in the placebo group was 7.6 days

Oral administration of DP resulted in an even better expressed antiviral effect. The results fully correlated with the stronger IFN-inducing activity

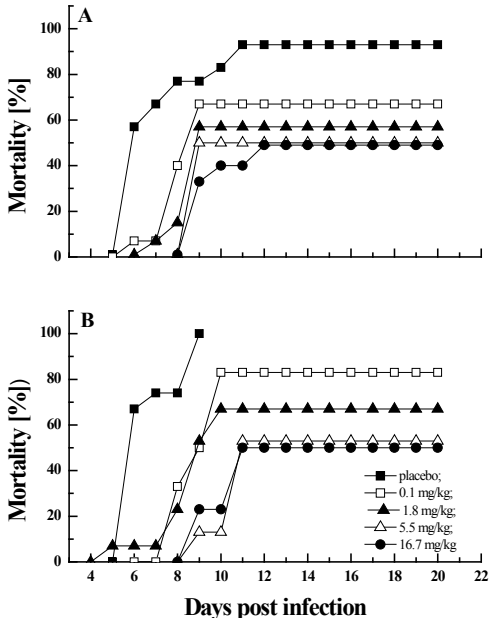

Fig. 1. Effect of DP in mice infected with alphavirus SFV: i. p. single administration 24 h before virus inoculation by 10 MLD_{50} (A) and 100 MLD_{50} (B)

of DP when administered per os. In infection with 5-10 LD_{50}, the antiviral effect of a single dose of DP depends on the time of its administration. When administered 24 h prior to infection, the optimal IFN-inducing doses, 12.5 and 25 mg/kg, were most effective (Fig. 2A). The percentage of surviving animals was 93% and 80%, respectively (70% mortality in the placebo group) and the MST was prolonged by 12.2 and 9.5 days, respectively. The dose-response curve retained its character when DP was administered 24 h after virus inoculation, although in that case the antiviral effect was noticeably reduced (Fig. 2B).

Fig. 2C shows the effect of the compound applied at the 72nd h after infection, i.e. at the middle of the latency period. It is noteworthy that DP in this case showed also marked efficiency, at doses of 50 and 100 mg/kg the protective rate being 50% and 80%, respectively, and the lengthening of the MST – 6.5 and 10.9 days. The dose of 12.5 mg/kg was found to be inactive. This unexpected result could be explained with some peculiarities of the development of the alphavirus infection and possibly by the more rapid attainment of the efficient IFN inducer concentration.

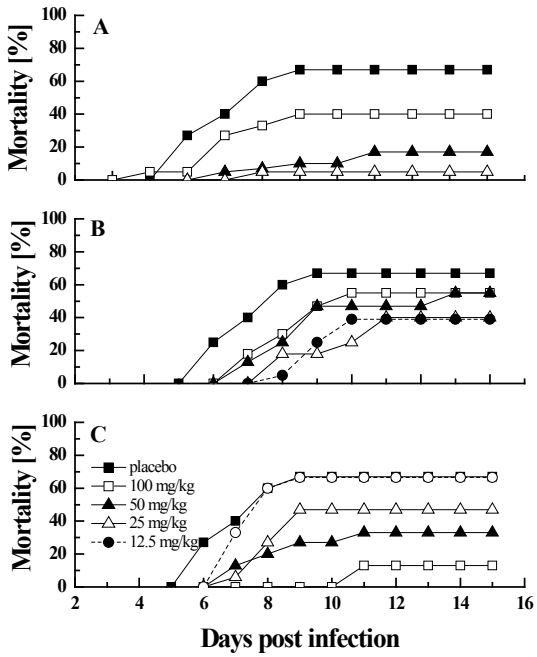

Fig. 2. Effect of DP in ice infected with alphavirus SFV: oral single administration of DP at virus infection with 10 MLD_{50} – 14 h prior (A), 24 h post (B) or 72 h post virus inoculation (C)

Effect of DP on experimental infections with influenza viruses A and B

Mice inoculated inranasally by 5-10 LD_{50} of influenza virus A/Puerto Rico/8/34 (H1N1) were treated *per os* with single DP doses – 100, 50 or 12.5 mg/kg 24 h prior to, 24 or 72 h post virus inoculation. We found a marked protective effect when the substance was applied 24 or 72 h after virus inoculation, and no distinct effect was registered when DP was administered prior to virus inoculation (Figures 3A, 3B, 3C).

This protective effect was approximately equal to that observed by Tonew et al. (1982) when DP was applied by oral route 5 times (-2, +2, +24, +48 and +72 h) at doses of 40 mg/kg, or 80 mg/kg (the first one divided in two daily intervals) in mice infected with influenza virus A/England/42/72 (H3N2).

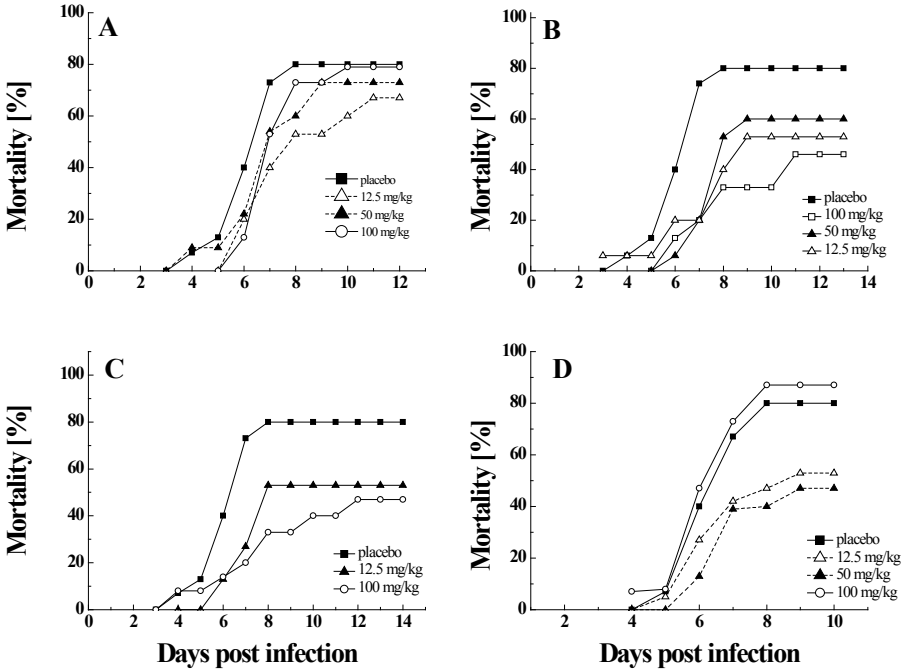

Fig. 3. Effect of DP in mice infected with influenza virus A/Puerto Rico/8/34 (H1N1), administered singly *per os* 24 h prior to (A), 24 post (B) or 72 h post (C) virus inoculation (5-10 MLD50). DP applied orally 24 h post infection with influenza virus B/Lee/40 (D).

DP also exhibited a protective effect in albino mice experimentally infected with influenza B/Lee/40, 10 LD_{50}, when administered 24 h after virus inoculation at the IFN-inducible concentration of 12.5-50 mg/kg (Fig. 3D). In this case, the substance was inactive when administered 72 h post virus inoculation, while a single dose administered 24 h prior to viral inoculation in a dose of 100 mg/kg resulted only in an extension of the survival time by 1.6 days.

Effect of DP in experimental HSV type 1-induced encephalitis

After single-dose i. p. administration of DP 24 h prior to HSV type 1/strain Lennett infection, DP was effective only at the optimal IFN-inducing dose of 16.7 mg/kg. The protective rate was 44.7% and the prolongation of the MST from 6.3 days in the placebo group attained 17.3 days in the DP treated group (Fig. 4A).

In experimental infection with HSV type 1/strain Leningrad 2, a certain protective effect was found only in a 50 mg/kg DP dose administered 24 h prior to virus inoculation. The effect at 16.7 mg/kg was not significant (Fig. 4B). Applied i. p. post virus inoculation, DP was ineffective (Fig. 4C).

Following single-dose oral administration of DP 24 h before and 24 or 72 h post virus inoculation (Leningrad 2 strain) (Fig. 5), a distinct protective effect was observed in a dose of 12.5 mg/kg (optimal IFN-inducing dose) - a decrease of mortality and prolongation of the MST by 4.7 days (when administered 24 h before infection) and 4 days (applied 24 h post infection), respectively, as well as certain delay of the MST by 7.5, 5.0 or 7.1 days (after administration 24 h prior to, and 24 or 72 h after virus inoculation, respectively).

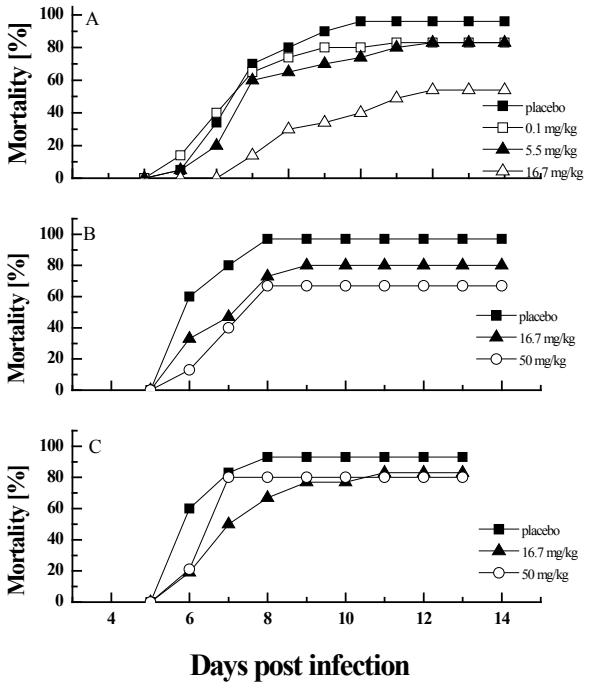

Fig. 4. Effect of DP single i. p. administration in mice with HSV type 1 (10 MLD50) –induced encephalitis: DP applied i. p. 24 h prior to (A) HSV-1 Lennett strain infection, (B) 24 h prior to HSV-1 Leningrad 2 strain virus infection; (C) 24 h after HSV-1 Leningrad strain virus inoculation

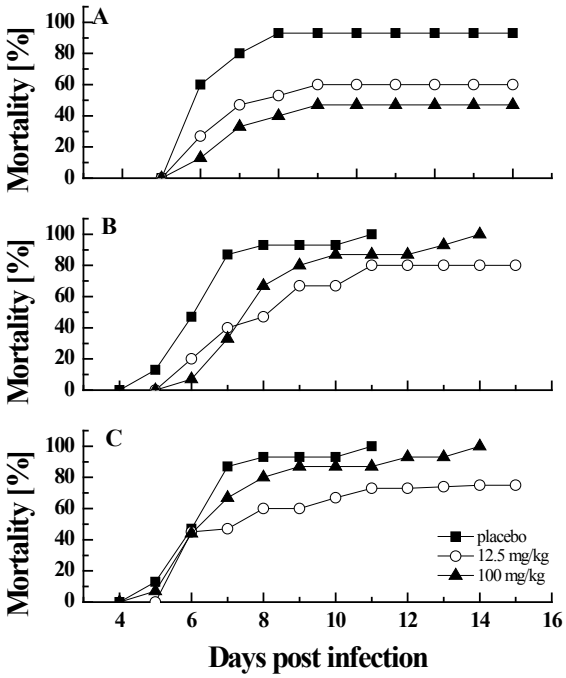

Fig. 5. Effect of DP single oral administration in mice with with HSV type 1 strtain Leningrad 2 (10 MLD50) – induced encephalitis: 24 h before (A), 24 h after (B) or 72 h post (C) virus inoculation

Effect of DP in experimental cutaneous infection with HSV type 1

Mice of C57Bl line, with a 5-day latency period from the 6th to the 10th day post infection with HSV type 1 strain Leningrad 2, developed a typical picture of cutaneous herpes simplex (Table 1). DP single i. p. administration of a single 50-mg/kg dose suppressed markedly the course of herpes simplex infection if the compound was introduced 24 h before or 72 h post virus inoculation. The development of local lesions (vesicles and crusts) was prevented in 37.7% of the animals treated, whereas in the placebo group only in 7.1% local lesions were not demonstrable. In the ill animals treated with DP, the infection had a weaker course and on the 16th day a recovery in all animals was recorded, while in the placebo group – in 42.9% of the animals only. Following IFN inducing administration, at the 72nd h a stronger antiviral effect in comparison with the experimental group treated 24 h prior to infection was observed. Even at the 8th day a recovery was recorded in 2/3 of the animals with manifested skin herpes, i.e. animals with no lesions were in sum 71.4% of the total number of mice, 35.7% being in the group treated 24 h before virus inoculation , and zero percentage in the placebo group.

DP applied in the 120th hour, i.e. at the end of the latency period did not influence the course of infection.

Table 1. Effect of dipyridamole (i.p. administration of a single daily dose of 50 mg/kg) on skin infection with herpes simplex virus type 1 (Leningrad 2 strain) in mice (C57BL)

Time of DP* administration	Skin lesions development (*total mice number) Days post virus inoculation														
	6th			7th			8th			9th			10th		
	+V	+Cr	n.l.	+V	+Cr	n.l.	+V	+Cr	n.l.	+V	+Cr	n.l.	+V	+Cr	n.l.
Placebo	100.0	0	0	100.0	0	0	76.9	23.1	0	69.3	30.7	0	0	53.8	46.2
-24 hr	0	35.7	41.7	16.6	41.7	41.7	16.6	41.7	41.7	16.6	41.7	0	0	100.0	64.3
+72 hr	0	35.7	0	23.1	76.9	0	23.1	76.9	0	23.1	76.9	0	0	100.0	64.3
+120 hr	100.0	0	0	100.0	0	0	63.6	36.4	0	45.5	54.5	0	0	33.3	66.7

*DP = dipyridamole; V = vesicles; Cr = crusts; n.l. = no lesions

Discussion and Conclusions

The results obtained from testing the antiviral activity of DP in experimental viral infections in mice indicate that this IFN inducer has manifested a pronounced protective effect by decreasing mortality and prolonging the MST. The effect was particularly well expressed in experimental infection with SFV in mice. DP was applied according to the IFN inducer scheme in single oral administration at the optimal IFN-inducing dose 24 h prior to lethal viral infection with SFV, 10-100 LD_{50}. In this mode of administration, DP reduced several times the morality of infected animals. A protective effect, though weaker, was found with single-dose administration 24 and 72 h after viral inoculation. A noticeable protective effect was also observed after i. p. administration at optimal IFN-inducing dose.

In experimental orthomyxovirus infection in mice (influenza A/PR8/34 (H1N1) and strain B/Lee/40) DP was most effective when administered 24 h after inoculation (10 LD_{50}), being lless effective

24 h prior to infection, and ineffective 72 h post infection. Our results are approximately the same as the results obtained by Tonew *et al*. (1982).

The identity of single- and multiple-dose effects of DP administration in influenza infections supports our argument that the antiviral effect of DP is due to its IFN-inducing activity.

A prominent protective effect occurs in experimental brain infection with HSV type 1 when DP is administered 24 h before viral inoculation (i. p. or oral route of administration). Later administration of the preparation reduces that effect. DP has a distinct protective effect in experimental cutaneous infection with HSV type 1, strain Leningrad 2, in albino mice when administered once 24 h before and 72 h after viral inoculation.

The antiviral effect of DP is due to its IFN-inducing activity. The characteristics of the IFN-inducing activity of DP *in vitro* and *in vivo* are the most convincing argumentation in this respect. The effective scheme in experimental viral infections is single-dose administration of DP at optimal IFN-inducing doses. A similar correlation between IFN-inducing and antiviral activity exists in other low-molecular-weight IFN inducers. IFN-induced DP could also affect the immune system (Koychev *et al*., 1985; MIhaylova *et al*., 1985). It is quite likely that, by stimulating the lymphoreticular system for IFN production, the substance actually stimulates other components of the immune system. Obviously, the protective effect of IFN may also include the effect it exerts on other systems, but that would require further detailed studies of its impact on the immune system.

The conclusions regarding the antiviral activity of DP in experimental viral infections in white mice are as follows:

(1) DP is an effective antiviral agent in experimental viral infections with SFV, HSV type 1, influenza A/Puerto Rico/8/34 (H1N1) and influenza virus B (Lee strain).

(2) The compound shows higher antiviral activity with a single administration at IFN-inducing doses: from 12.5 mg/kg to 100 mg/kg by oral administration, and from 5.5 mg/kg to 50 mg/kg by i. p. route of administration.

(3) The antiviral effect of DP is most clearly expressed when administered 24 h before, and 24 h and 72 h after viral inoculation in experimental infections with SFV and HSV type 1, at infection with influenza A(H1N1) - 24 h or 72 h post-infection, and in influenza B/Lee - 24 h after infection.

These data are new proofs of the hypothesis that the antiviral effect of DP is due to its IFN-inducing capacity. Besides, these results may serve as a basis for determination of the optimal scheme of treatment by DP in the clinics.

References

Bańkowski, A., K. Filczak, M. Korbecki, E. Tonew (1981). The effect of dipyridamole on the multiplication of vaccinia virus in RK13 cells. *Acta Virol.* **25**: 256.

Galabov, A. S., A. U. Itkes, M. Mastikova, V. L. Tunitskaya, E. S. Severin (1985). Dipyridamole-induced interferon production in mouse peritoneal leucocytes. *Biochem. Int.* **11**: 591-598.

Galabov, A. S., K. Kisyova, M. Mastikoiva, K. Metodiev (1987). Deviations in the immune profile and the interferon response in lung diseases *Klinicheskaya medicina* **12**: 89-92 (in Russian).

Galabov, A. S., M. Mastikova (1982). Dipyridamole is an interferon inducer. *Acta Virol*. **26**: 137-147.

Galabov, A. S., M. Mastikova (1983). Interferon-inducing activity of dipyridamole in mice. *Acta Virol*. **27**: 356-358.

Galabov, A. S., M. Mastikova (1984a). Dipyridamole as an interferon inducer in man. In: Physiology and Pathology of Interferon System (L. Borecky, V. Lackovic, eds)/ Contributions to Oncology vol. 20/S. Karger, Basel, pp. 278-284.

Galabov, A. S., M. Mastikova (1984b). Dipyridamole induces interferon in man. *Biomed. Pharmacother*. **38**: 412-413.

Galabov, A. S., M. Mastikova (1985). Dipyridamole: inductor de interferon en humanos. *Compendium de Investigaciones Clinicas Latinoamericanas* **2**: 37-39.

Grigoryan, S. S., A. S. Galabov, F. I. Ershov (1989). Induction of interferon by dipyridamole in cultures of human leukocytes and in autoimmune and oncological diseases in humans. In: Interferon-89 (S. B. Prozorovskii et al., eds). USSR AMS, N. F. Gamaleya RIEM, Moscow, pp. 149-152 (in Russian).

Ilyenko, V. I. (1977). Methods of testing and evaluation of antiviral activity of chemical compounds towards influenza virus Ed. by All-Union Research Institute of Influenza, Leningrad, Russia, (in Russian).

Konstantinov, K., A. Galabov, M. Mastikova (1989). Interferon response to dipyridamole in lupus erythematosus patients. *British J. Dermatol*. **121**: 59-63.

Korbecki M, A. Bankowski A, K. Filczak, A. Klimek (1985). Dipyridamole as an inhibitor of vaccinia virus replication (Article in Russian). *Mol. Gen. Mikrobiol. Virusol.* **1**: 29-32.

Koychev, Ch., S. G. Mihaylova, G. Guencheva, A. S. Galabov (1985). Effects of dipyridamole on rosett-forming cells in in vitro and in vivo experiments. *C. r. Acad. bulg. Sci*. **38**: 409-412.

Kuzmov, K., A. S. Galabov, Kh. Radeva, M. Kozhukharova, K. Milanov, P. Doseva-Runevska, M. Mastikova (1985). Epidemiological trial of the prophylactic effectiveness of dipyridamole, an interferon inducing agent, with respect to influenza and acute respiratory diseases. *Zhurnal Mikrobiol. Epidemiol. Immunol.* **6**: 26-30, (in Russian).

Michaylova, S. G., T. Ts. Kutsarova, A. S. Galabov, G. P.

Guencheva (1985). Effects of dipyridamole on phagocytic activity of mouse peritoneal macrophages. *C. r. Acad. bulg. Sci.* **38**: 413-415.

Oehring, H., J. Schmidt (1978). Zur antiviralen Wirkung von Dypiridamol auf Rhinoviren. *Z. Ges. Hyg. Grenzgeb*. **24**: 447.

Snoeck, R., G. Andrei, J. Balzarini, D. Reymen, E. De Clercq (1994). Dipyridamole potentiates the activity of various acyclic nucleoside phosphonates against varicella-zoster virus, herpes simplex virus and human cytomegalovirus. *Antivi. Chem. Chemother*. **5**: 312-321.

Tonew, E., M. K. Indulen, D. R. Dzeguze (1982). Antiviral action of dipyridamole and its derivatives against influenza virus. *Acta Virol*. **26**: 125-129.

Tonew, M., D. Dzeguze (1977). Dipyridamol, an inhibitor of mengovirus replication in FL and L cells. *Chemotherapy* **23**: 149-158.

Tonew, M., E. Tonew, E. Mentel (1977). The antiviral activity of dipyridamole. *Acta Virol*. **21**: 146-150.