Institute of Microbiology[1], Bulgarian Academy of Sciences, Nat. Cent. Infect. Paras. Dis.[2], Department of Virology, Sofia, Bulgaria

Mycophenolic acid as acyclovir partner for combined inhibition of herpes viruses

S. Pancheva[1], I. Roeva[1], D. Dundarova[2] and M. Remichkova[1]

It has recently been demonstrated that the reduction of dNTP pools size in virus-infected cells could result in an increased antiviral effectiveness of some selective antiviral nucleoside analogues [1–4]. In our recent studies, it has been shown that dGTP and dTTP could be considered "key metabolites" responsible for a potentiation of the combinations of acylovir-ribavirin and bromovinyldeoxyuridine-methotrexate, respectively, against herpes simplex virus infections [5, 6]. According to our concept in combined drug chemotherapy of virus infections target to nucleotide metabolism, the use of a partner drug which decreases the pool of the target nucleotide in virus-infected cells is very perspective. In this study investigating the antiherpes activity of acyclovir (ACV) in combination with mycophenolic acid (MPA), an active immunosuppressive agent, this concept could be confirmed again.

MPA inhibits non-competitively the IMP-dehydrogenase, the rate-controlling enzyme of the de novo biosynthesis of GMP, thus depleting cellular pools of GTP [7, 8]. ACV acts as a competitive inhibitor of the natural metabolite/substrate dGTP in its binding to viral DNA polymerase [9]. The decrease in the cellular pool of dGTP could give a better chance to the competitor to interact with the target enzyme. In the present studies it is demonstrated that MPA significantly enhances the antiviral activity of ACV against HSV-1 and HSV-2 in cell cultures of human embryonic skin-muscle fibroblasts (HESMF), and pseudorabies virus (PRV) in CEF cells. In a previous communication [7] preliminary results were described about the enhancing effect of MPA on anti-HSV-1 activity of ACV. It was established that MPA at a concentration of 100 μM causes 50% cytotoxicity to uninfected HESMF cells. MPA was examined in very low concentrations (0.32–0.04 μM), which by themselves had no effect on the replication of HSV-1 and HSV-2 in culture of HESMF cells. The activity of ACV in the presence of MPA was evaluated against HSV-1 and HSV-2 using a cytopathic effect (CPE)-inhibition method. The dose-response curves obtained are presented in the Fig. It is evident that MPA markedly reduces the HSV-1 and HSV-2-induced (CPE) of ACV. At the highest concentration of MPA of 0.16 μM (0.05 μg/ml) the 50% effective concentration (ED_{50}) of ACV against HSV-1 decreases 30-fold following combination with MPA (from 1.0 μg/ml to 0.03 μg/ml). As can be seen from the Fig. the DRCs of ACV shift to the left to lower ED_{50} values by fixed doses of MPA that delineated a potentiating interaction of the two compounds [11]. Similar results were obtained by the yield reduction method. The synergistic inhibition activity of ACV + MPA on the replication of different herpesvirus strains is demonstrated in the Table. The combination of ACV (1.8 μM), which alone decreased the virus titer of HSV-1 21 times, with MPA (0.16 μM) causes a 4000-fold reduction of the virus yield. As it is seen from the Table the potentiating effect of MPA on the activity of ACV is stronger against PRV in CEF cells. ACV (8.9 μM) and MPA (0.16 μM), which alone reduced the virus titer 17 and 7 times, respectively, suppressed in combination the PRV replication 6×10^4 times, indicating a high degree of synergism.

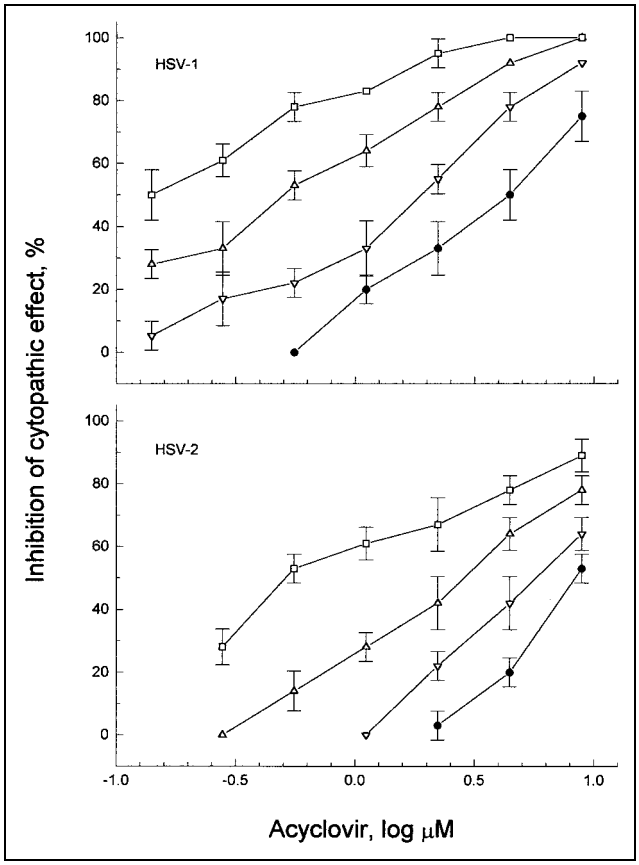

Fig.: Dose-response curves of inhibition of virus-induced cytopathic effect in HESMF cells by ACV alone (●) and in the presence of 0.16 μM (□), 0.08 μM (△) and 0.04 μM MPA (▽)

Table: Potentiating effect of MPA on the inhibitory activity of ACV against HSV-1 and HSV-2 replication in HESMF cells and PRV replication in CEF cells

Virus	Drugs	Conc. μM	Virus titer log $CCID_{50}$/0.1 ± SD	Fold titer reduction[a]
HSV-1	Control		5.95 ± 0.42	1
	ACV	1.8	4.61 ± 0.35	21
	MPA	0.16	5.29 ± 0.34	4.5
	ACV/MPA	1.8/0.16	2.33 ± 0.29	4.1×10^3
HSV-2	Control		4.67 ± 0.16	1
	ACV	1.8	3.78 ± 0.25	8
	MPA	0.32	4.33 ± 0.29	2
	ACV/MPA	1.8/0.32	2.30 ± 0.25	2.4×10^2
PRV	Control		6.28 ± 0.25	1
	ACV	8.9	5.05 ± 0.42	17
	MPA	0.16	5.42 ± 0.39	7
	ACV/MPA	8.9/0.16	1.5 ± 0.27	6×10^4

[a] Fold titer reduction = virus titer of no-drug control divided by virus titer in presence of drug(s). Data are mean values for 3 separate experiments

The inhibition of herpes simplex virus replication by the combination could be reversed when infection was carried out in excess of guanosine (Guo). In the presence of 8 μM Guo (50-fold higher concentration than that of MPA) the normal virus yield of HSV-2 at 48 h of incubation post infection was recovered. As Guo is converted within the cell into dGTP, it could be assumed that the potentiating effect of MPA on the antiviral effect of ACV is due to the reduced level of dGTP content, because of the strong inhibitory effect of MPA on IMP-DH activity.

Taking in view also the immunosuppressive action of MPA, this combination could have potential therapeutic benefit against herpesvirus infections developing very often in patients with organ transplantations, autoimmune diseases and other immune depending illness.

Experimental

ACV was provided by Burroughs Wellcome Co., MPA originated from Sigma. HSV-1 strain DA and HSV-2 were received from Dr. S. Dundarov, NCIPD, MA, Sofia. HSV-1 and HSV-2 were cultivated in cultures of diploid embryonic human skin-muscle fibroblasts (HESMF). Pseudorabies virus (PRV) strain Bucharest was grown in primary chick embryo fibroblasts (CEF). The antiviral activity was determined by measurement of viral CPE reduction in 96-well microplates and by the yield reduction method in 24-well microplates. The experiments were carried out in confluent cell monolayers infected with 100 $CCID_{50}$ in 0.1 ml. The drugs alone and in combination were added after 1 h of virus adsorption and the cells were than incubated for 48 h at 37 °C. The viral cytopathic effect was scored on 0–4 basis, with 4 representing total destruction. Virus yields in samples were determined by titration in microplate cultures of cells, and expressed in $CCID_{50}$/0.1 ml. Cytotoxicity was monitored by the trypan blue exclusion method. No cytotoxicity of the ACV and MPA combination in the antiviral assays was observed in the concentrations used.

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Prof. S. Pancheva, PhD
Institute of Microbiology
Bulgarian Academy of Sciences
113 Sofia
Bulgaria