LETTER TO THE EDITOR

ANTIHERPETIC ACTIVITY OF ACYCLOVIR IS POTENTIATED BY MYCOPHENOLIC ACID

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It has been demonstrated that the reduction in the intracellular content of natural metabolites could make their direct competitors more effective at the enzyme level (1–4). In our recent studies, we have shown that dGTP and dTTP could be considered “key metabolites” responsible for synergism of the combinations of acyclovir-ribavirin and bromovinyldeoxyuridine-methotrexate against herpes simplex virus (HSV) infections (5, 6). We think that in combined drug chemotherapy of virus infections targeted to nucleotide metabolism, the use of a partner drug which decreases the pool of the target nucleotide in virus-infected cell is very perspective. In this study, we present results which confirm the above mentioned concept by combining acyclovir (ACV), a well known antiviral drug, with mycophenolic acid (MPA), an effective inhibitor of inosine monophosphate (IMP) dehydrogenase.

Similarly to ribavirin, MPA inhibits non-competitively IMP-dehydrogenase, the rate-controlling enzyme of the de novo biosynthesis of GMP, thus depleting cellular pools of GTP and dGTP (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 this study, we demonstrate that MPA significantly enhances the antiviral activity of ACV against HSV-1 in human embryonic skin-muscle fibroblast (HESKF) cultures and that this inhibition is partially reversed by excess of guanosine (Guo). As Guo is converted within the cell to dGTP, our observations suggest that the reduction in dGTP level accounts for the potentiating effect of MPA on the antiviral activity to ACV.

ACV was provided by Dr. G. Elion, Burroughs Wellcome Co., Research Triangle Park, NC) and MPA originated from Sigma. HSV-1 strain DA was provided by Dr. S. Dundarov, Institute of Infectious and Parasitic Diseases, Bulgarian Medical Academy, Sofia. The virus was grown in diploid HESMF cultures. The activity of the combination of MPA and ACV was evaluated against HSV-1 in HESMF cells by the virus yield reduction method. The experiments were carried out on confluent cell monolayers in tubes infected with 100 CCID₅₀ in 0.1 ml. The drugs alone and in combination were added after 1 hr of virus adsorption and the cells were then incubated for 48 hrs at 37°C. Virus yields in samples (pools of 4 tube cultures) were determined by titration in microplate cultures of HESMF cells and expressed in CCID₅₀/0.1 ml.

The figure shows data on the antiviral activity of a combination of MPA in non-effective concentration of 0.16 µmol/l (0.05 µg/ml) and ACV in concentration of 1.8 µmol/l (0.4 µg/ml), which alone reduced the virus yield 21 times. This combination of MPA + ACV caused a 4000-fold reduction of the virus yield which indicates a significant potentiation of the anti-HSV-1 activity of ACV. Each column represents a mean value from three separate experiments ± SD. Similar results were obtained with HSV-2 replication in the same cells (data not shown).

Studies of the reverse effect of Guo on the inhibitory action of the combination ACV + MPA were carried out by using three different concentrations of Guo (Guo₁ = 8 µmol/l; Guo₂ = 80 µmol/l; Guo₃ = 800 µmol/l) administered simul-

Abbreviations: ACV = acyclovir; HESKF = human embryonic skin-muscle fibroblast; HSV = herpes simplex virus; Guo = guanosine; IMP = inosine monophosphate; MPA = mycophenolic acid.
Simultaneously with ACV and MPA. The rate of reverse effect was dependent on the concentration of Guo. A partial (8.9%) recovery of the virus yield 48 hrs p.i. was observed in the presence of 800 µmol/l Guo while no reversal of the antiviral action was found by 8 µmol/l Guo.

In conclusion, these data can be considered an additional example of the molecular strategy in the antiviral combined chemotherapy, in which an optimal selection of combinations of drugs with known mechanisms of action is at present possible.

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References