THE ANTIHERPES EFFECT OF ACYCLOVIR IS POTENTIATED BY THE INHIBITORS OF INOSINE 5'-MONOPHOSPHATE DEHYDROGENASE - RIBAVIRIN, MICOPHENOLIC ACID AND MIZORIBINE

M. Remichkova
Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

ABSTRACT
Inhibitors of cell enzyme Inosine 5'-Monophosphate (IMP) dehydrogenase – Ribavirine (Rbv), Micophenolic acid (MPA) and Mizoribine (MZR) have potential application as antiherpes agents in combination with acyclovir (ACV). The effectivity of action of ACV on herpesvirus replication is improved after decreasing of the concentration of dGTP as a result of IMP dehydrogenase inhibition. The antiviral effect of the combination between Rbv, MPA or MZR and ACV is reversiblle. Thus, the combinations have been shown to be effective against various herpesviruses: herpes simplex virus type 1 (HSV-1), herpes simplex virus type 2 (HSV-2), varicella-zoster virus (VZV), human cytomegalovirus (HCMV) and pseudorabies virus (PRV) in vitro and/or in vivo.

Introduction
The infections caused by herpesviruses are serious health problem. The members of the herpesviruses caused a number of diseases in humans and animals, as fever blisters, veneral herpes, herpetic keratoconjunctivitis, chickenpox, shingles, infectious mononucleosis and encephalitis. Same herpesviruses also cause cancer. Preferable therapeutic drug for treatment of several herpesviruses infections is ACV.

Acyclovir (9-[2-Hydroxyethoxy)methyl ] guanine) is acyclic guanosine analogue, which was synthesised 20 years ago from dr. Elion /1/. The drug has high efficacy and specificity of action in treatment of herpesvirus infections. The active form of the compound is ACV-triphosphate. It is obtained after transformation, by specific viral thymidine kinase (TK), to ACV-monophosphate, whereas di- and tri-phosphates are products of action of cell enzymes. The ACV-triphosphate form competing substrate dGTP for viral DNA polymerase.

ACV has been used as a precursor for synthesis of a wide series of synthetic derivatives: valciclovir, famciclovir, penciclovir, ganciclovir, lobocavir and several acyclic/carbocyclic guanosine analogues (A-5021, synguanol, D- and L-cyclohexenyl G/.

ACV was administered locally, orally and intravenously for therapy of herpes simplex virus, varicella and zoster, as well as cytomegalovirus infections. Significant success has been obtained in the therapy of herpes encephalitis by early diagnosis and treatment with ACV /2/.

The administration of ACV has been attended with emergence of resistance in patients with herpesvirus infections /3/. It has been reported about ACV-resistant herpes simplex, zoster and cytomegalovirus strains
in immunocompromised patients /4, 5/. The studies have shown, that ACV resistance is due mainly to a mutation, resulting to HSV strain unable to produce viral TK, or rarely to a mutation in the polymerase gene.

The inhibitory effect of ACV on herpesvirus replication could be changed by a combination with drugs, inhibitors of key enzymes from the cell metabolism. Marked enhancement of the antiviral activity of ACV was achieved after combination with inhibitors of IMP dehydrogenase (Figure).

IMP dehydrogenase / IMPDH; EC 1.1.1.205 / is the rate-limiting enzyme in the novo pathways of the purine metabolism. The enzyme catalyzes NAD-dependent conversion of Inosine monophosphate (IMP) to Xantosine monophosphate (XMP). In cells infected with herpesvirus the concentration of the dGTP is reduced as result of IMP dehydrogenase inhibition and the possibility of triphosphate form of ACV to interact with viral DNA polymerase is increased. Wherever, significant inhibition of the herpesvirus replication is achieved.

Rbv, MPA, MZR, Tiazofurin, EICAR and VX-497 are the most studied inhibitors of IMP dehydrogenase/6, 7, 8/.

It has been demonstrated that Rbv, MPA and MZR markedly enhanced the antiviral activity of ACV.

**Ribavirin**

Ribavirin (1-β-D-ribofuranosyl-1, 2, 4-triazole-3-carboxamide) is a synthetic nucleoside structurally related to pyrazofurin (pyrazomycin), guanosine and xanthosine. The drug has been known as antiviral agent and also has moderate antitumor and immunosuppressive effects /9/.

Rbv has activity against many RNA viruses, including respiratory sinclital virus, influenza A and B viruses, parainfluenza virus and rhinovirus /10, 11/. Oral Rbv was used in combination with interferon-α for treatment of chronic hepatitis C infection in patients with compensated liver disease /12/. Rbv has been effective for therapy of pneumonitis caused by adenoviruses /13/.

Rbv acts also on replication of DNA viruses, like Poxviruses, alone or in combination with Cidofovir /14/.

The inhibitory activity of Rbv on viral

---

**Figure.** Mechanism of action of the combination ACV /ACG-TP/ and inhibitors of IMP dehydrogenase on herpesvirus replication.
replication depends on the intracellular conversion to Rbv-5'-monophosphate, -diphosphate and triphosphate. Rbv-5'-triphosphate functions as inhibitor of viral RNA polymerase. Rbv-5'-monophosphate acts on viral RNA and DNA synthesis through prevention of the formation of guanine monophosphate, respectively GTP, after IMP dehydrogenase inhibition. In common, the specificity of action of Rbv is to a high degree depending on the kind of viral infection.

Rbv was found to be very effective on herpesvirus replication in combination with ACV /15/. The potentiating effect of Rbv on inhibitory activity of ACV has been examined in vitro and in vivo. It has been obtained about 63% inhibition of the virus yield in treatment of HSV-1 and PRV in cell cultures. The antiherpetic effect of the combination has been restored after adding of Guanosine with ACV and Rbv /16/. The combination was applied successfully in the treatment of herpetic keratoconjunctivitis in rabbits /17/.

Micophenolic acid
Micophenolic acid is a reversible, noncompetitive inhibitor of IMP dehydrogenase, produced by Penicillium sp. /18/. MPA is an immunosuppressive, antitumor and antiviral agent. The ester prodrug of MPA, micophenolate mophetil has been developed as immunosuppressor and approved for the prevention of acute rejection in kidney and cardiac transplantations in combination with steroids and cyclosporin A. There is evidence that MPA inhibits Poxviruses and Hepatitis B virus in vitro /19, 20/. Similarly to Rbv, MPA was applied mainly in combination with other viral inhibitors. It has been shown, that MPA in combination with purine nucleoside analogues was used for treatment of HIV infections /21/. The inhibitory activity of ACV on the replication of HSV-1 and HSV-2 in cell cultures was significantly increased by the combination with MPA. It has been demonstrated, that the combination between MPA and ACV markedly inhibit replication of HSV-1 and HSV-2 in HESMF /human embrionoc skin-muscle fibroblasts/. It has been achieved a 4000-fold reduction of the virus yield by combination of 0.16 µM MPA with 1.8 µM ACV. The antiviral effect of the combination was restored by added guanosine. Moreover, the combination was used successfully for treatment of PRV infection in CFC /primary chick embryo fibroblasts//22, 23/.

There are data that MPA markedly potentiates the antiviruc activity of ACV, pencyclovir /PCV/, ganciclovir /GCV/ on HSV-1, HSV-2, VZV, CMV in vitro and in vivo. In mice infected with HSV-1 or HSV-2 was obtained complete protection after 2-times treatment with ACV and MPA /24/. The potentiating effect of MPA was manifested also in combination with other guanosine analogues: lobocavir against HSV-1, HSV-2, TK-HSV-1, HCMV; H2G against HSV-1, HSV-2, VZV, TK-VZV and A-5021 against HSV-1, HSV-2 and TK-HSV-1 /25/.

Mizoribine
Mizoribine /4-carbamoyl-β-D-ribosil imidazolium-5-olate/ also known as bredinin is a competitive specific inhibitor of IMP dehydrogenase, isolated from the soil fungus Eupenicillium brefeldianum /26/. The drug is an imidazole nucleoside with a structure as Rbv and has immunosuppressive, anticancer and antivirus activities. It has been used in clinics in Japan for prevention of rejection of organ transplantation and for therapy of autoimmune diseases, reumatoid arthritis and lupus nephritis. In mammalian cells MZR metabolizes and then inhibits the target enzyme. The conversion to 5'-monophosphate form is carried out by the cell enzyme adenosine kinase /27/.

It has been shown that MZR inhibits Candida albicans and several viruses in vitro: Vaccinia virus, RS virus and Influ-
The inhibitory effect on herpesvirus replication was not found, but it was examined that MZR markedly potentiates the antivirus activity of ACV in vitro. The combination ACV/1.1 µM/ and MZR/154 µM/ causes complete reduction of the virus yield, when the infection was carried out in primary human fibroblasts/31, 32/. The antiherpes effect of the combination was restored by exogenous guanosine. The relative studies of the effect of the combinations Rbv/ACV and MZR/ACV showed that Rbv and MZR have the same effect on HSV replication/33/.

Conclusions
The inhibitory activity of ACV on herpesvirus replication is enhanced by the combination with Rbv, MPA or MZR. The combinations are useful to preventing drug resistance, as well as to increasing the effectiveness of herpesvirus therapy. Moreover, concerning immunosuppressive activity of MPA and MZR this might be significant for development of more effective treatment of herpesvirus infections that often occur in patients with organ transplantation.

REFERENCES
9, 221-235.


