Human herpesvirus type 1 (HHV-1) commonly known as Herpes simplex virus type 1 (HSV-1) belongs to the Alphaherpesvirinae subfamily of Herpesviridae family.

Acyclovir (ACV) is the first selective inhibitor of alphaherpesviruses. It was discovered by G. Elion and H. Scheafer in 1974 and became the treatment of choice for HHV-1 and HHV-2 infections. Until now, ACV is the most widely prescribed antiviral drug in the world. The drug is similar in structure to a DNA component – guanine deoxyriboside and is activated by viral thymidine kinase (TK) to its active form. TK is a 376-amino acid protein, encoded by UL23 gene. In infected host cells ACV is phosphorylated by thymidine kinase to acyclovir monophosphate, host cellular enzymes, further, phosphorylate this drug and convert it to its active triphosphate form. The final product leads to irreversible inhibition of viral DNA synthesis by chain termination of viral DNA strands. ACV is well-tolerated, side effects are not frequent. Resistance of HHV-1 to ACV is related to mutations within UL 23 (thymidine kinase; TK) and UL30 (DNA polymerase) genes. ACV-resistant HHV infections are rare in immunocompetent hosts (from 0.1 to 1.0%). In immunocompromised patients, the prevalence of strains with reduced susceptibility to ACV depends on the reason of immunosuppression and ranges from 3.5 to 30% (1-5). The first clinical cases of acyclovir-resistant herpes simplex viruses were described in 1982, shortly after the initial use of intravenously administered acyclovir. It is well known that prolonged use of ACV is the most important risk factor for the selection of resistant strains, but drug-resistant strains have also been isolated in the absence of a known history of acyclovir treatment (1).

The second-line antiviral agents for HHV-1 infections is foscarnet (FOS) and cidofovir (CDV). Foscarnet is an organic analogue of inorganic pyrophosphate; directly inhibits DNA polymerase by blocking the pyrophosphate binding site and preventing cleavage of pyrophosphate from deoxynucleotide triphosphates.

Oral bioavailability is low, so foscarnet must be administered intravenously. The most common adverse effects of FOS are: seizures, fever, nausea, diarrhea, headache, leucopenia, nephrotoxicity and metabolic derangements.

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Resistance to FOS can be developed quickly after a relatively short period of use. Most FOS resistant clinical HSV isolates contain single base substitutions in conserved regions II, III, VI, or VII and in a non-conserved region of the DNA pol gene.

Cidofovir is an acyclic phosphonate nucleotide analogue that has demonstrated potent, broad-spectrum activity against double-stranded DNA viruses. Only 2–26% of CDV is absorbed after oral administration, so cidofovir should be administered intravenously in the clinical management of patients. Because the most important adverse reaction during the therapy of cidofovir is nephrotoxicity, CDV is contraindicated in patients with moderate to severe preexisting renal dysfunction. Resistance is now rare, mostly due to mutations within the viral DNA pol gene (1, 4, 6, 7).

Common occurrences of serious HHV infections in both immunocompetent and immunocompromised patients, a small number of available antiviral drugs and the emergence of drug resistance necessitate research for new antiviral agents that work through different mechanisms of action or biologically active products which can stimulate immune responses against herpesviral infection. Another possible option is to search a new applications of available drugs and co-administration them to elicit additive or even synergistic effect (1, 7-11).

The immune system can be manipulated non-specifically by immunomodulation. There are many well-known substances capable of enhancing host defence mechanisms to provide protection against infection. One of them are inosine pranobex (isoprinosine) and interferons (12).

Inosine pranobex exerts an antiviral effects which are both direct and secondary to immunomodulatory activity. The mechanism of action in human body is still unclear but numerous studies have demonstrated that this drug exhibit pleiotropic effect. Inosine pranobex can augment the production of cytokines such as interleukin-1 (IL-1) and interleukin-2 (IL-2). It increases the production of interleukin 12 (IL-12), INF-γ and decreases interleukin-3 (IL-3) and interleukin-4 (IL-4) production in vitro. Inosine pranobex normalizes the cell-mediated immunity stimulating T-cell differentiation into cytotoxic T cells and T-helper cells and increasing cytokine production. It also increases the humoral immune response by stimulating the differentiation of B-lymphocytes into plasma cells and by enhancing antibody production. The agent potentiates neutrophil, monocyte, macrophage chemotaxis and phagocytosis. Inosine pranobex augments NK activity and inhibits viral replication as well. In in vivo studies inosine pranobex reduced severity of symptoms and shortened the duration of some viral infections. It has been found to be useful in treatment of several viral diseases such as herpes simplex, rhinovirus infections and herpes zoster, influenza, genital warts, EBV infection (12-18).

Combination of inosine pranobex and IFN-α appear to be effective treatment of the persistent meases complication of the central nervous system – subacute sclerosing panencephalitis (SSPE) (13, 19, 20). It has been reported that inosine pranobex possesses a weak anti-HIV-1 activity in human peripheral blood mononuclear cells (PBMC) and H9 cells and restores in vitro the impaired T-helper cells from patients with AIDS-related complex (21). Inosine pranobex is virtually non-toxic. After prolonged treatment with high doses nausea is occasionally observed (13).

Interferons are antiviral proteins (cytokines) that can be formulated from purified, natural, human interferons or produced using recombinant DNA techniques. They constitute a family of cytokines capable of inducing an antiviral state within a cells. They are potent and pleiotropic immune modulators and can inhibits replication of many viruses. The mechanism of action of interferons is complex – they can interfere with almost any stage of viral attack.

About 55 years ago, the first antiviral drug idodeoxyuridine (IDU) was described. This compound becomes the first antiviral drug to be licensed for use in the treatment of HHV infections. Now, there are more than 50 licensed antiviral compounds, but half of them are used for the treatment of HIV infection. The discovery of effective antiviral agents has been facilitated by advances in the fields of chemistry, molecular biology and virology. Key issues in the development of novel antivirals are the emergence of resistant strains. The development of new drugs effective against human viral diseases has proven to be both difficult and time-consuming. Some alternative may be to optimize the efficacy and selectivity of existing antiviral drugs or combining them with other well known agents (7, 9, 12).

The aim of this study was to evaluate in vitro inhibition of three strains of HHV-1 replication by inosine pranobex and interferon-α. In particular, the study was focused on the problem if compounds may act specifically against different strains of human herpesviruses type 1.

**EXPERIMENTAL**

**Compounds**

Inosine pranobex (isoprinosine) was kindly provided by Gedeon Richter (Poland). Inosine pra-
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In vitro inhibition of HHV-1 replication by inosine pranobex (IP) is a synthetic compound formed from the p-acetamido benzoate salt of N-N dimethylamino-2-propanol, and inosine in a 3 : 1 molar ratio. IP is highly soluble in water and chemically stable. The concentrations used in tissue culture studies were non-toxic and ranged from 50 to 400 µg/mL. Concentrations of IP were chosen according to the toxicity examination. Interferon used in the studies was IFN-α-2a (Roche) at final concentrations 100 and 1000 IU/mL. Doses of IFN-α were chosen based on a search of the literature (22).

Viruses

The viral strains used in this study were: standard (laboratory, reference) strain of human herpesvirus type 1 McIntyre (HHV-1MC) – sensitive to acyclovir and two resistant to acyclovir clinical isolates (HHV-1H3a, HHV-1f12k). HHV-1 H3a was obtained from an orofacial lesions from a patient with hematologic disorders. HHV-1f12k was obtained from - previously treated with aciclovir - woman with recurrent genital herpes.

Susceptibility to antiviral drugs (ACV and CDV) was assessed in vitro by phenotypic and genotypic assays. The yield reduction assay (YRA) which evaluates the ability of the compound to inhibit virus production in cell culture was applied in phenotypic tests. The Reed-Muench statistical method was used to determine the 50% end point (IC50).

HHV-1MC is sensitive to acyclovir, the IC50 value was 0.68 µg/mL. Clinical isolates are resistant to both acyclovir and cidofovir. The IC50 value of ACV for both clinical strains of HHV-1 were > 250 µg/mL, and IC50 of CDV for HHV-1H3a and HHV-1f12k were > 500 µg/mL. The thymidine kinase (TK) gene (UL23) sequence (352 bp) from HHV-1 clinical isolates was amplified and detected using the PCR. Using this genotypic methods we found that one of the phenotypically resistant strains (HHV-1H3a) shows changes in the gene encoding thymidine kinase, which is required for the phosphorylation of nucleoside analogs.

Tissue cultures

Antiviral activity of tested compounds have been compared in various cell lines. Viruses were propagated in human larynx carcinoma cells HEp-2 (ATCC, CCL-23) and primary human lung fibroblasts HEL 299 (ATCC, CCL-137). HEL 299 cells were cultured in Dulbecco’s modified Eagles medium (DMEM, Sigma-Aldrich) containing 10% fetal bovine serum (Gibco Life Technologies, UK) and 1% penicillin/streptomycin antibiotics (Gibco Life Technologies, UK). HEp-2 cells were grown and maintained in Eagle’s medium essential medium (Biomed, Poland). The cells were cultured at 37°C in a humidified atmosphere containing 5% CO2.

Cytotoxicity assay

This test was performed with HEp-2, HEL 299 and A549 (human lung adenocarcinoma epithelial cell line; ATCC, CCL185). The cytotoxicity of the inosine pranobex (at doses of 25-800 µg/mL) and IFN-α (at concentration of 100 and 1000 IU/mL) against three cell lines was assessed visually using light, inverted microscopy Olympus CK2 (Olympus Corp., Germany) and by the MTT colorimetric assay. The assay determines the ability of viable cells to convert a soluble tetrazolium salt [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) into an insoluble formazan precipitate. Cells were incubated in 96-microwell plates. After incubation of cells for 24 h, inosine pranobex and/or IFN-α were added (at doses given above) and cultured further for 24 and 48 h. In controls the cells were cultured without any of the tested drugs. All experiments were performed in triplicates. Absorbance values of examined samples was read spectrophotometrically at a wavelength of 490 nm on a reader (Reader 230, Organon Technica Turnhout, Belgium).

Antiviral assay

The antiviral effects of IFN-α and inosine pranobex were assessed in vitro by phenotypic assays. The antiviral activity of inosine pranobex was tested using series of non-toxic concentrations (50-400 µg/mL) diluted in an assay medium. IFN-α was used at final concentrations: 100 and 1000 IU/mL. To investigate the antiviral activity, cell cultures in microwell plates were infected with HHV-1 strains (0.01 TCID50/cell) for 60 min at 37°C. After the absorption, the inoculum was aspirated and fresh culture medium containing IP, IFN-α or both agents were added. Next, the cells were incubated for 48 h. The yield reduction assay (YRA) which evaluates the ability of the compounds to inhibit virus production in cell culture was applied. The cytopathic effect of the virus was evaluated by means of light, inverted microscopy, 48 h after infection of cultures with viruses. The Reed-Muench statistical method was used to determine the 50% end point (IC50), which was the lowest concentration of the tested drugs that reduce the viral infections of the control to a 50%.

Antiviral effect was estimated according to the reduction of the herpesvirus titer in the presence of
compounds with the controlled one. TCID<sub>50</sub>/mL was calculated, it is a median tissue culture infective dose; that amount of a pathogenic agent that will produce pathological change in 50% of cell cultures inoculated. Each analysis was performed at least in triplicate. The results were statistically evaluated using Pearson’s correlation method to measure the correlation between the doses of IP and the titers of viruses. Calculated titers of viruses were evaluated by using a two-tailed Student’s t-test to compare two small sets of quantitative data (TCID<sub>50</sub>, the measure of infectious virus titers).

The Wilcoxon signed rank sum test was also used to analyze differences between the INF/IP treated infected cells and the control group (infected cells treated only IP) in terms of efficacy to reduction of viral titer. A value of p < 0.05 was considered as statistically significant.

RESULTS

Cytotoxity assay

In this study, the effect of IP and IFN-α was assessed in in vitro experiments. Three different cell lines were exposed to varying concentrations of each compound (IP at doses of 25-800 µg/mL and IFN-α at concentration of 100 and 1000 IU/mL) for 48 h. The cytotoxicity was measured microscopically and by the MTT assay. There were no morphological changes in cell cultures treated with IP and IFN-α in the examined concentrations. Visual assessment of the cell monolayer showed no toxic effect in the presence of IP and IFN-α. MTT cytotoxicity assay confirmed earlier microscopic observations. The viability of cells in the presence of tested compounds was higher than 95%.

The compounds at all tested concentrations were nontoxic to A549, HEp-2 and HEL 299 cell lines. For this reason the TC<sub>50</sub> (the toxic drug concentration which caused the reduction of viable cell numbers by 50%) and SI (selectivity index; CC<sub>50</sub> to IC<sub>50</sub> value) were not calculated.

Antiviral assay

Increasing concentrations of inosine pranobex (50–400 µg/mL) produced progressively greater inhibition of HHV-1<sub>MC</sub> and HHV-1<sub>H3a</sub> replication following infection of HEp-2 and inhibition the growth of HHV-1<sub>MC</sub> in HEL 299 cell cultures (Pearson’s correlation, p < 0.05).

A summary of the inhibitory effects of IP on virus replication in HEL 299 and HEp-2 cells is shown in Table 1. When IFN-α alone was added to HHV-1 infected cells, only a weak antiviral effect was detected even at concentration as high as 1000 IU/mL. INF-α in the concentration 1000 IU/mL caused only about 1 log reduction in titers of HHV compared to that of the controls. Results were similar in the case of reference and clinical HHV strains (Fig. 2).

The inosine pranobex, when used at a concentration of 400 µg/mL, markedly inhibited the replication of HHV-1<sub>MC</sub> in HEL 299 cell line. The combined treatment with IP (400 µg/mL) and INF-α (1000 IU/mL) significantly inhibited multiplication of the HHV-1<sub>MC</sub> in HEL 299 cell culture as compared to the control where the virus has not been exposed to IP and/or IFN-α (Student’s t-test, p < 0.05). The average viral titer was inhibited by about 3 logarithms in comparison to the control and below 1 logarithm in comparison to the viral titer after exposition to the IP (400 µg/mL) alone.

Otherwise, the reduction of the HHV-1<sub>MC</sub>, HHV-1<sub>H3a</sub> and HHV-1<sub>F12k</sub> titers in HEp-2 cell line after applying IP 400 µg/mL and α-INF (1000 IU/mL), in comparison to the viral titer in the con-

| Table 1. The titer of the viral stock (TCID<sub>50/mL</sub>) in cultures of HHV-1 infected cells incubated with inosine pranobex (IP). |
|---|---|---|---|---|
| Cell line | HEL299 | HEp-2 | HEp-2 | HEp-2 |
| Virus | HHV-1<sub>MC</sub> | HHV-1<sub>H3a</sub> | HHV-1<sub>MC</sub> | HHV-1<sub>F12k</sub> |
| TCID<sub>50/mL</sub> | | | | |
| control | 7.50E+05 | 9.30E+05 | 9.30E+05 | 9.30E+05 |
| IP 50 µg/mL | 1.10E+05 | 9.30E+05 | 9.30E+05 | 9.30E+05 |
| IP 100 µg/mL | 1.10E+05 | 7.50E+05 | 7.50E+05 | 1.60E+05 |
| IP 200 µg/mL | 1.60E+04 | 1.70E+05 | 1.60E+05 | 1.60E+05 |
| IP 400 µg/mL | 1.10E+03 | 5.90E+04 | 5.90E+04 | 7.50E+04 |
| Pearson’s r | -0.92 | -0.92 | -0.92 | -0.77 |
| p value | p < 0.05 | p < 0.05 | p < 0.05 | p = 0.1 |
In vitro inhibition of HHV-1 replication by inosine...

control, were reduced by more than 1.5 logarithms (Student’s t-test, p < 0.05). The reduction of the HHV-1MC, HHV-1H3a, and HHV-1F12K titer in HEp-2 cell line after treatment with IP (400 µg/mL) and IFN-α (1000 IU/mL), in comparison to the viral titer after applying of inosine pranobex 400 µg/mL, was reduced by below 1 logarithm (Fig. 2).

Combination of IP and IFN-α display higher efficacy than either treatment alone and may increase therapeutic effect without augmenting toxic effects. Results are statistically significant only for HHV-1MC treated simultaneously with maximal doses of IP and INF-α in HEL-299 culture (Wilcoxon signed-rank test, p < 0.05).

The antiviral activity of the tested compounds was also analyzed on the basis of IC50 values (Table 2). The IC50 values of IP against HHV-1MC standard strain were 513.56 µg/mL (HEL 299) and 886.86

Figure 1. Reduction of viral titers in cultures of HEL 299 and HEp-2 cells incubated with inosine pranobex (IP) 400 µg/mL and/or IFN-α 100 IU/mL.

Figure 2. Reduction of viral titers in cultures of HEL 299 and HEp-2 cells incubated with inosine pranobex (IP) 400 µg/mL and/or IFN-α 1000 IU/mL.
µg/mL (HEp-2). The IC₅₀ value of IP alone against the clinical strain HHV-1₃H₃a was 883.98 µg/mL (HEp-2). In contrast, dose-related response for clinical isolate HHV-1₃H₃a in HEp-2 cell line was weaker. The IC₅₀ value as high as 1047.65 µg/mL was observed.

When infected cells were treated with IFN-α and IP simultaneously, an enhanced antiviral activity was found. Application of IFN-α (1000 IU/mL) with IP after infection of HEL 299 and HEp-2 with HHV-1Mc reduced the IC₅₀ to 378.71 µg/mL and 445.51 µg/mL, respectively.

The combination of 1000 IU/mL IFN-α and IP also resulted in enhanced anti-HHV- clinical strains activity but less efficiently. IC₅₀ were reduced to 923.07 µg/mL (HHV-1f12k) and 810.81 µg/mL (HHV-1H3a) (Table 2). The strongest inhibitory effect on viral replication was observed after combined of addition maximal doses of IP and IFN-α to the HHV-1Mc infected HEp-2 culture. IC₅₀ has been reduced by approximately 50%.

DISCUSSION AND CONCLUSIONS

Infectious diseases are known since ancient time. The development of antiviral agents has progressed slowly. Numerous compounds have been evaluated for antiviral activity in cell cultures and in animals studies, but only a small number of them have been approved for human use (23-25). There are some anti-herpes medications; however, there is no cure for herpes infection. Antiviral drugs can reduce the frequency, duration and severity of outbreaks, as well as asymptomatic shedding of virus (1). Idoxuridine (5-iodo-2-deoxyuridine) was the first medication to be approved (1962) against herpesviral infection. Because of systemic toxicity, its use was restricted to the topical use in the herpes keratitis (26). The another drug used in the treatment of primary and secondary keratoconjunctivitis caused by HHV-1 was trifluorothymidine (5-trifluoromethyl-2-deoxyuridine, TFT). The next achievement was the introduction of vidarabine for treatment of herpes encephalitis - a serious life-threatening disease. Now, because of the many side effects and adverse reactions it is used only in keratitis caused by HHV-1 which has not responded to topical idoxuridine or when toxic or hypersensitivity reactions due to idoxuridine have occurred (24, 26). Acyclovir (ACV, 9-(2-hydroxyethoxy)methyl guanine) was the first selective inhibitor of HHV replication. Having one of the most remarkable safety profiles of any antiviral agents, acyclovir became the treatment of choice for all HHV-1 infections. Unfortunately, shortly after the initial use of intravenously administered acyclovir the first clinical cases of resistant strain were reported. Another anti-herpesviral drugs (cidofovir and foscarnet) have a low bioavailability so they are preferentially administered intravenously and cause a lot of serious adverse reactions during the therapy (1, 27). No vaccine is currently available to prevent herpes infections.

Consequently, there is a great need for the development of new, safe, orally bioavailable drugs. The design and synthesis of anti-herpesvirus agents that possess a mechanism of action different than currently used compounds seems to be especially desirable (9, 10, 28-30). It is well known that the course of viral infection, the severity of symptoms, response to treatment and the ability to select resistant mutants depends on the patient’s immunocompetence.

<table>
<thead>
<tr>
<th>Cell line →</th>
<th>HEL 299</th>
<th>HEp-2</th>
<th>HEp-2</th>
<th>HEp-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virus →</td>
<td>HHV-1Mc</td>
<td>HHV-1Mc</td>
<td>HHV-1H3a</td>
<td>HHV-1F12k</td>
</tr>
<tr>
<td>IP (50-400 µg/mL)</td>
<td>IC₅₀</td>
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<td>886.86</td>
<td>883.98</td>
</tr>
<tr>
<td>IC₅₀ (max-min.)</td>
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<td>982.05-805.53</td>
<td>1000.16-787.84</td>
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<tr>
<td>IP (50-400 µg/mL)/IFN-α 100 IU/mL</td>
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<td>884.87</td>
<td>883.54</td>
</tr>
<tr>
<td>IC₅₀ (max-min.)</td>
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<td>1212.87-678.34</td>
<td>1077.20-739.86</td>
<td>1469.60-757.27</td>
</tr>
<tr>
<td>IP (50-400 µg/mL)/IFN-α 1000 IU/mL</td>
<td>IC₅₀</td>
<td>378.71</td>
<td>445.51</td>
<td>810.81</td>
</tr>
<tr>
<td>IC₅₀ (max-min.)</td>
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<td>525.35-380.91</td>
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<td>1143.38-649.46</td>
</tr>
</tbody>
</table>
Immunomodulators can be real adjuncts to established therapeutic rules offering a novel approach for the treatment of infectious diseases (31).

In the conducted experiments, the ability to reduce the cytopathic effect caused by the reference acyclovir susceptible and clinical acyclovir resistant HHV-1 strains (showing different mechanisms of resistance) was investigated. This study was focused on the question if the antiviral effect is enhanced through the addition of IFN-α. Biron was the first reviewer who stated clearly that IFN-α plays an important role in shaping downstream innate and adaptive immune responses to viral infections (32). Cytokine-induced antiviral mechanisms are differentially expressed in different cell lines. The degree of inhibition of HHV-1 replication in vitro depends on the cell line, and an examined strain (22).

The available evidences indicate that IFN-α severely inhibits the replication of several viruses in vitro. Some studies indicate that replication of HHV-1 is poorly reduced in cultured cells. However, results of in vivo studies with IFN receptor-null mice and HHV-1 strains illustrated the importance of IFN-α in controlling early stages of infection in animals (22, 34). Mossman mentioned that the absence of interferon receptors resulted in disseminated infection and severe disease. For this reason further in vivo drug interaction studies based on in vitro evaluation are necessary.

So far, a combination of IFN-α and isoprinosine has brought positive results in the treatment of subacute sclerosis panencephalitis (SSPE). SSPE is a persistent and chronic encephalitis secondary to measles virus infection that causes widespread demyelination of the central nervous system. Such combined therapy is still recommended because of theoretical synergistic effects (35). Like Mossman et al. and Kuczer et al. we have observed that the degree of inhibition of viral replication in vitro depends on the cell line, and examined strain (10, 22). IP at a concentration of 400 µg/mL exerts the strongest inhibitory effect on the replication of HHV-1 in HEL 299 cell line. It seems that the best choice for an in vitro susceptibility testing can be the primary human lung fibroblasts.

During the past decades, cytokine-based therapies have been developed. To date, multiple cytokines with Th1-stimulating properties, such as IFN-α, IL-2 and IL-12, have been evaluated, alone or in combination for the treatment of bladder cancer (30, 36). Our studies have confirmed the observations made by other authors that the effect of interferon can be augmented after the simultaneous addition of other drugs (31, 35-38).

The enhanced antiviral activity was found when infected cells were treated with IFN-α and IP simultaneously. When IFN-α was added to HHV-1-infected cells alone, only a weak antiviral activity was detected even the concentration was as high as 1000 IU/mL. Results are statistically significant only for HHV-1MC treated simultaneously with maximal doses of IP and INF-α in HEL-299 culture.

The IC₅₀ values were most strongly reduced when IFN-α (1000 IU/mL) and IP were added. The most potent inhibitory effect on viral replication was observed when combination of maximal doses of IP and IFN-α to the HHV-1MC were used. In this case, IC₅₀ has been reduced by 26.3 and 49.8%, respectively, in HEL 299 and HEP-2 cell lines. The synergistic effect of IFN-α in combination with other drug is well known. Falzarano et al. identified a potential therapeutic approach against nCoV (novel β corona virus) isolate combining IFN-α and ribavirin. Either treatment alone reduced virus replication by at least 1 log or as much as 4 logs in susceptible cell lines. Moreover, when both agents were combined, the efficacy was reached at lower concentrations (37). Mosa et al. suggest that combination of interferon and ribavirin should be considered in immunocompromised patients and probably also in cases of diffuse and/or complicated papillomatoses unresponsive to first- and second-line treatments (38).

It is well known that acyclovir is very effective in suppressing HHV-1 infections in the early stages of disease if the strain is ACV-sensitive (1, 6, 39). Inosine pranobex enhances T cell proliferation and activity and is also approved for treatment of HHV-1 and HHV-2 infections, though it is significantly less active than other more traditional antiherpes drugs.

Increased understanding of the antiviral immune mechanisms and the limited number of drugs which have been approved for medical use against HHV in the last few decades encourage to supplement HHV-infected patients with isoprinosine and passive immunotherapy. Immunotherapy may be especially beneficial in patients infected with viral strains resistant to currently known antiviral drugs.

REFERENCES


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