Product Monograph

PrZOVIRAX®
Acyclovir Cream, Manufacturer’s Standard

Antiviral Agent
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Actions and Clinical Pharmacology

ZOVIRAX® (acyclovir), a synthetic acyclic purine nucleoside analog, is a substrate with a high degree of specificity for herpes simplex and varicella-zoster specified thymidine kinase. Acyclovir is a poor substrate for host cell-specified thymidine kinase. Herpes simplex and varicella-zoster specified thymidine kinase transform acyclovir to its monophosphate which is then transformed by a number of cellular enzymes to acyclovir diphosphate and acyclovir triphosphate. Acyclovir triphosphate is both an inhibitor of, and a substrate for, herpesvirus-specified DNA polymerase. Although the cellular α-DNA polymerase in infected cells may also be inhibited by acyclovir triphosphate, this occurs only at concentrations of acyclovir triphosphate which are higher than those which inhibit the herpesvirus-specified DNA polymerase. Acyclovir is selectively converted to its active form in herpesvirus-infected cells and is thus preferentially taken up by these cells. Acyclovir has demonstrated a very much lower toxic potential in vitro for normal uninfected cells because: 1) less is taken up; 2) less is converted to the active form; 3) cellular α-DNA polymerase has a lower sensitivity to the action of the active form of the drug. A combination of the thymidine kinase specificity, inhibition of DNA polymerase and premature termination of DNA synthesis results in inhibition of herpesvirus replication. No effect on latent non-replicating virus has been demonstrated. Inhibition of the virus reduces the period of viral shedding, limits the degree of spread and level of pathology, and thereby facilitates healing. During suppression there is no evidence that acyclovir prevents neural migration of the virus. It aborts episodes of recurrent herpes due to inhibition of viral replication following reactivation.
Indications and Clinical Use

ZOVIRAX® (acyclovir) Cream 5% is indicated for the topical management of initial episodes of genital herpes simplex infections. The prophylactic use of this preparation has not been established.

In the treatment of genital herpes, appropriate examinations should be performed to rule out other sexually transmitted diseases. Therapy should begin as early as possible after the start of an infection.

Two multicentre, double-blind, placebo-controlled studies were performed with ZOVIRAX® Cream in immunocompetent patients with initial genital herpes. The cream was applied for up to 10 days or until healing had occurred. Results showed that ZOVIRAX® Cream significantly reduced the duration of viral shedding, the formation of new lesions, the time to crusting and healing of lesions, and the duration of pain.

Whereas cutaneous lesions associated with herpes simplex infections are often pathognomonic, Tzanck smears prepared from lesion exudate or scrapings may assist in the diagnosis. Positive cultures for herpes simplex virus offer the only absolute means for confirmation of the diagnosis.

Contraindications

ZOVIRAX® (acyclovir) Cream 5% is contraindicated for patients who develop hypersensitivity or chemical intolerance to acyclovir, valacyclovir or any of the components of the formulation, such as propylene glycol.

Warnings

ZOVIRAX® (acyclovir) Cream 5% is intended for topical use only and should not be used in the eye.

Precautions

General

ZOVIRAX® (acyclovir) Cream 5% is not recommended for application to mucous membranes such as the mouth or vagina.
The recommended dosage, frequency of application and duration of treatment of ZOVIRAX® Cream should not be exceeded (see DOSAGE AND ADMINISTRATION).

There exist no data, at this time, which demonstrate that the use of ZOVIRAX® Cream will prevent transmission of infection to other persons.

Since most cutaneous herpes simplex virus infections result from reactivation of latent virus, it is unlikely that ZOVIRAX® Cream will prevent recurrence of infections when applied in the absence of signs and symptoms. ZOVIRAX® Cream should not be applied in an attempt to prevent recurrences; application should commence only at the earliest prodromal sign of disease onset.

Although clinically significant viral resistance associated with the use of ZOVIRAX® Cream has not been observed, this possibility exists. (See VIROLOGY).

**Sexual Function/ Reproduction**

There is no information on the effect of acyclovir oral formulations on human female fertility. In a study of 20 male patients with normal sperm count, oral acyclovir administered at doses of up to 1g per day for up to six months has been shown to have no clinically significant effect on sperm count, motility or morphology.

**Nursing Mothers**

Acyclovir, when given systemically, is known to be excreted into human milk. No information is available on levels of acyclovir which may appear in breast milk after administration of ZOVIRAX® Cream. Caution should be exercised when acyclovir is administered to a nursing mother.

**Use in Pregnancy**

Teratology studies carried out to date in animals have been negative in general. However, in a non-standard test in rats, there were fetal abnormalities such as head and tail anomalies, and maternal toxicity; since such studies are not always predictive of human response, ZOVIRAX® should not be used during pregnancy unless the physician feels the potential benefit justifies the risk of possible harm to the fetus. The potential
for high concentrations of acyclovir to cause chromosome breaks in vitro should be taken into consideration in making this decision.

A post-marketing acyclovir pregnancy registry has documented pregnancy outcomes in women exposed to any formulation of ZOVIRAX®. The registry findings have not shown an increase in the number of birth defects amongst ZOVIRAX® exposed subjects compared with the general population, and any birth defects showed no uniqueness or consistent pattern to suggest a common cause.

**Use in Children**

Safety of use of ZOVIRAX® Cream in children has not been established.

**Drug Interaction**

Clinical experience has identified no interactions resulting from topical or systemic administration of other drugs concomitantly with ZOVIRAX® Cream.

**Adverse Reactions**

Because ulcerated genital lesions are characteristically tender and sensitive to any contact or manipulation, patients may experience discomfort upon application of ZOVIRAX® (acyclovir) Cream 5%. The table below shows the number of initial genital herpes patients who reported adverse reactions in the two controlled clinical trials:
### Adverse Reaction

<table>
<thead>
<tr>
<th>Adverse Reaction</th>
<th>ZOVIRAX® <em>(n=54)</em></th>
<th>Placebo <em>(n=47)</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Burning/stinging on application</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Rash</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Itching</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Retention of urine</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Meningism</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Paronychia</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total No. (%) of patients</strong></td>
<td><strong>6 (11%)</strong></td>
<td><strong>15 (32%)</strong></td>
</tr>
</tbody>
</table>

**Observed During Clinical Practice:** Based on worldwide clinical practice experience in patients treated with ZOVIRAX® Cream, the adverse events most commonly reported include contact dermatitis, application site reaction, eczema, allergic reaction, pain, and rash.

Less common events include pruritus, skin discoloration, urticaria, vesiculobullous rash, and facial edema.

There have been very rare reports of immediate hypersensitivity reactions including angioedema with topical acyclovir.

### Symptoms and Treatment of Overdosage

Overdosage by topical application of ZOVIRAX® (acyclovir) Cream 5% is unlikely because of limited transcutaneous absorption.

### Dosage and Administration

Apply ZOVIRAX® (acyclovir) Cream 5% liberally to the affected area 4 to 6 times daily for up to 10 days. A sufficient quantity should be applied to adequately cover all lesions. A finger cot or rubber glove should be used while applying ZOVIRAX® Cream in order to prevent autoinoculation of other body sites or transmission of infection to other persons. **Therapy should be initiated as early as possible following onset of signs and symptoms.**
Pharmaceutical Information

Drug Substance

Acyclovir:

Molecular Formula: $\text{C}_8\text{H}_{11}\text{N}_5\text{O}_3$

Molecular Weight: 225.2 Daltons

Chemical Name: 9-[(2-hydroxyethoxy)methyl]guanine

Other Names: Acycloguanosine

Description: Acyclovir is a white, crystalline powder with a maximum solubility in water of 1.3mg/mL at 25°C.

Composition

Each 1 gram of ZOVIRAX® Cream 5% contains 50mg acyclovir and the non-medicinal ingredients propylene glycol, paraffin, cetostearyl alcohol, poloxamer, sodium lauryl sulfate.

Storage Conditions

ZOVIRAX® Cream 5% should be stored between 15°C and 25°C and kept dry.

Dosage Forms

ZOVIRAX® Cream 5% is available in tubes of 5g.
Virology

The quantitative relationship between the in vitro susceptibility of herpes simplex and varicella-zoster viruses to acyclovir and the clinical response to therapy has not been established in man, and virus sensitivity testing has not been standardized. Sensitivity testing results, expressed as the concentration of drug required to inhibit by 50% the growth of virus in cell culture (ID$_{50}$), vary greatly depending upon the particular assay used, the cell type employed, and the laboratory performing the test. The ID$_{50}$ of acyclovir against HSV-1 isolates may range from 0.02μg/mL (plaque reduction in Vero cells) to 5.9-13.5μg/mL (plaque reduction in green monkey kidney [GMK] cells). The ID$_{50}$ against HSV-2 ranges from 0.01μg/mL to 9.9μg/mL (plaque reduction in Vero and GMK cells, respectively).

Using a dye-uptake method in Vero cells, which gives ID$_{50}$ values approximately 5 - to 10-fold higher than plaque reduction assays, 1417 HSV isolates (553 HSV-1 and 864 HSV-2) from approximately 500 patients were examined over a 5-year period. These assays found that 90% of HSV-1 isolates were sensitive to ≤0.9μg/mL acyclovir and 50% of all isolates were sensitive to ≤0.2μg/mL acyclovir. For HSV-2 isolates, 90% were sensitive to ≤2.2μg/mL and 50% of all isolates were sensitive to ≤0.7μg/mL of acyclovir. Isolates with significantly diminished sensitivity were found in 44 patients. It must be emphasized that neither the patients nor the isolates were randomly selected and, therefore, do not represent the general population. Most of the less sensitive HSV clinical isolates have been relatively deficient in the viral thymidine kinase (TK). Strains with alterations in viral TK or viral DNA polymerase have also been reported. Prolonged exposure to low concentrations (0.1μg/mL) of acyclovir in cell culture has resulted in the emergence of a variety of acyclovir-resistant strains.

The ID$_{50}$ against VZV ranges from 0.17-1.53μg/mL (yield reduction, human foreskin fibroblasts) to 1.85-3.98μg/mL (foci reduction, human embryo fibroblasts [HEF]). Reproduction of EBV genome is suppressed by 50% in superinfected Raji cells or P3HR-1 lymphoblastoid cells by 1.5μg/mL acyclovir. CMV is relatively resistant to acyclovir with ID$_{50}$ values ranging from 2.3-17.6μg/mL (plaque reduction, HEF cells) to 1.82-56.8μg/mL (DNA
hybridization, HEF cells). The latent state of the genome of any of the human herpesviruses is not known to be sensitive to acyclovir.

**Resistance**

Prolonged exposure of herpes simplex virus (HSV) to subinhibitory concentrations (0.1μg/mL) of acyclovir in cell culture has resulted in the emergence of a variety of acyclovir resistant strains. The emergence of resistant strains is believed to occur by "selection" of naturally occurring viruses with relatively low susceptibility to acyclovir. Such strains have been reported in pre-therapy isolates from several clinical studies.

Two resistance mechanisms involving viral thymidine kinase (required for acyclovir activation) have been described. These are: (a) selection of thymidine-kinase-deficient mutants that induce little or no enzyme activity after infection, and (b) selection of mutants possessing a thymidine kinase of altered substrate specificity that is able to phosphorylate the natural nucleoside thymidine but not acyclovir. The majority of less susceptible viruses arising in vitro are of the thymidine-kinase-deficient type which have reduced infectivity and pathogenicity and less likelihood of inducing latency in animals.

However, an acyclovir-resistant HSV infection in an immunosuppressed bone marrow transplant recipient on extended acyclovir therapy was found to be due to a clinical isolate which had a normal thymidine kinase but an altered DNA polymerase. This third mechanism of resistance involving herpes simplex virus DNA polymerase is due to the selection of mutants encoding an altered enzyme, which is resistant to inactivation by acyclovir triphosphate.

Varicella Zoster virus appears to manifest resistance to acyclovir via mechanisms similar to those seen in herpes simplex virus.

However, limited clinical investigation has revealed no evidence of a significant change in in vitro susceptibility of varicella zoster virus with acyclovir therapy, although resistant mutants of this virus can be isolated in vitro in a manner analogous to herpes simplex virus. Analysis of a small number of clinical isolates from patients who received oral acyclovir or placebo for acute herpes zoster suggests that in vivo emergence of resistant varicella zoster virus may occur infrequently. Prolonged acyclovir
treatment of highly immunocompromised patients with acquired immunodeficiency syndrome and severe varicella zoster virus may lead to the appearance of resistant virus.

Cross-resistance to other antivirals occurs \textit{in vitro} in acyclovir-resistant mutants. Herpes simplex virus mutants which are resistant to acyclovir due to an absence of viral thymidine kinase are cross-resistant to other agents which are phosphorylated by herpesvirus thymidine kinase, such as bromovinyldeoxyuridine, ganciclovir and the 2'-fluoropyrimidine nucleosides, such as, 2'-fluoro-5-iodoarabinosyl-cytosine (FIAC).

The clinical response to acyclovir treatment has usually been good for patients with normal immunity from whom herpes simplex virus having reduced susceptibility to acyclovir has been recovered either before, during or after therapy. However, certain patient groups, such as the severely immunocompromised (especially bone marrow transplant recipients) and those undergoing chronic suppressive regimens have been identified as being most frequently associated with the emergence of resistant herpes simplex strains, which may or may not accompany a poor response to the drug. The possibility of the appearance of less sensitive viruses must be recognized when treating such patients, and susceptibility monitoring of clinical isolates from these patients should be encouraged.

In summary, the quantitative relationship between the \textit{in vitro} susceptibility of herpes simplex and varicella-zoster viruses to acyclovir and the clinical response to therapy has not been clearly established in man. Standardized methods of virus sensitivity testing are required to allow more precise correlations between \textit{in vitro} virus sensitivity and clinical response to acyclovir therapy.
Pharmacology

Dermal Absorption

Two studies were conducted to determine the percutaneous absorption of acyclovir cream.

In the first study, acyclovir (ACV) in dimethyl sulfoxide (DMSO) was evaluated for the treatment of cutaneous herpes simplex virus infection in guinea pigs and compared with ACV in polyethylene glycol (PEG). When compared with infection sites treated with the vehicle alone, ACV-DMSO produced a greater percent reduction than did ACV-PEG in the number, area and virus titer of the lesions.

The second study investigated penetration through excised human and guinea pig skin of ACV formulated in three different vehicles: PEG, DMSO, and modified aqueous cream (MAC). Results showed that ACV-MAC and ACV-DMSO penetrated through human and guinea pig skin at a faster rate than ACV-PEG.

Toxicology

Acute Toxicity Studies

Adult Mice, Rats and Rabbits: The acute toxicity of acyclovir was determined as follows:

<table>
<thead>
<tr>
<th>Species</th>
<th>Sex</th>
<th>Route</th>
<th>LD50 (mg/kg)</th>
<th>95% Conf. Level</th>
<th>Signs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>M</td>
<td>Oral</td>
<td>&gt;10 000</td>
<td>-</td>
<td>None</td>
</tr>
<tr>
<td>Rat</td>
<td>M</td>
<td>Oral</td>
<td>&gt;20 000</td>
<td>-</td>
<td>None</td>
</tr>
<tr>
<td>Mouse</td>
<td>M</td>
<td>IV</td>
<td>405</td>
<td>-</td>
<td>Ataxia Depression</td>
</tr>
<tr>
<td>Rat</td>
<td>M</td>
<td>IV</td>
<td>&gt;600</td>
<td>-</td>
<td>None</td>
</tr>
<tr>
<td>Mouse</td>
<td>M</td>
<td>IP</td>
<td>1 454</td>
<td>1323-1662</td>
<td>Sedation</td>
</tr>
<tr>
<td>Mouse</td>
<td>F</td>
<td>IP</td>
<td>999</td>
<td>670-1364</td>
<td>Sedation</td>
</tr>
<tr>
<td>Rat</td>
<td>M</td>
<td>IP</td>
<td>1 305</td>
<td>512-1733</td>
<td>Sedation</td>
</tr>
<tr>
<td>Rat</td>
<td>F</td>
<td>IP</td>
<td>1 210</td>
<td>504-1580</td>
<td>Sedation</td>
</tr>
<tr>
<td>Rabbit</td>
<td>M/F</td>
<td>Dermal</td>
<td>&gt;2 (g/kg)</td>
<td>-</td>
<td>None</td>
</tr>
</tbody>
</table>

Neonatal, Immature, and Adult Rats: Groups of 10 male and 10 female Charles River CD (Sprague-Dawley) rats were given single large doses (5 different dose levels) of a solution (pH 11.0) of acyclovir by subcutaneous injection when they were 3, 10, 28 and 71 days of age. They were
observed for 14 days after treatment and LD50 values were calculated by the Litchfield and Wilcoxon method. This study was done to determine if age at exposure affects the acute toxicity of acyclovir; there was no evidence that young rats were more sensitive than older rats to the acute toxic effects of acyclovir.

<table>
<thead>
<tr>
<th>Age When Treated</th>
<th>LD50 (mg/kg body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
</tr>
<tr>
<td>3 Days</td>
<td>1070</td>
</tr>
<tr>
<td>10 Days</td>
<td>790</td>
</tr>
<tr>
<td>28 Days</td>
<td>678</td>
</tr>
<tr>
<td>71 Days</td>
<td>650</td>
</tr>
</tbody>
</table>

There was no apparent relationship between length of survival after treatment and age at which treatment was given. Clinical signs for the rats treated at 3 and 10 days of age included red and purple cutaneous blisters, blue areas, scabs, scars, necrotic and sloughed skin, open wounds, body tremors and alopecia. Decreased activity, lacrimation, closed eyelids, red-brown or brown material around the eyes, nose and mouth, ataxia, prostration, body tremors, urine stains around the abdomen or genital area, scabbed or necrotic areas and alopecia were observed in rats treated at 28 and 71 days of age.

**Dermal Tolerance Studies**

Acyclovir Cream was evaluated in guinea pigs for sensitizing potential. The cream produced no signs of dermal sensitization.

Rabbits were treated 3 times a day for 21 consecutive days with acyclovir cream. The rabbits did not show signs of treatment-related discomfort; erythema at the treatment sites was barely perceptible in virtually all cases.

Acyclovir cream was applied at a dosage of 0.5mL/site to the backs of rabbits and the primary irritation index was calculated at 72 hours post-treatment. Acyclovir cream was found to be nonirritating.

Guinea pigs were treated with 0.4 mL doses of acyclovir cream, applied to the flank area. No signs of skin irritancy were noted.

**Mutagenicity and Other Short-Term Studies**

Acyclovir has been tested for mutagenic potential in a number of in vitro systems: cultured L5178Y mouse lymphoma cells (3 loci); cultured Chinese hamster ovary (CHO) cells (3 loci); Ames Salmonella (plate assay); Ames Salmonella (preincubation modification); Rosenkrantz E. coli polA⁺/polA⁻
DNA repair assay; and the yeast *S. cerevisiae*, D-4. Also, the drug has been tested in the BALB/C-3T3 Neoplastic Transformation Assay, in the C3H/10T ½ Neoplastic Transformation Assay and for clastogenicity in cultured human lymphocytes. All assays were done both in the presence and absence of exogenous mammalian metabolic activation except for the cell transformation tests and the human lymphocyte cytogenetic assay. *In vivo*, acyclovir has been examined in a mouse dominant lethal assay, and for clastogenicity in rat and Chinese hamster bone marrow.

*In vitro*, acyclovir was negative in all microbial assays; it was also negative at the HGPRT locus and the Ouabain-resistance marker in the mouse lymphoma system; and in the C3H/10T ½ assay for transformation. It was significantly positive at the highest dose tested in the BALB/C-3T3 cell transformation assay; it gave a moderately positive response at high concentrations at the TK locus in the mouse lymphoma assay and caused chromosomal breakage in human lymphocytes at high concentrations. *In vivo*, no cytogenetic effects were noted at up to nephrotoxic doses (100mg/kg) in rats or Chinese hamsters; at higher doses (500 and 1000mg/kg), chromosome damage was seen in Chinese hamster bone marrow. Summaries of the various assay results are as follows:

**Microbial:** Acyclovir was tested for mutagenic activity in the Ames Salmonella plate assay; in a preincubation modification of the Ames assay; in the Rosenkrantz *E. coli* polA+/polA- DNA repair assay; and in the eukaryote *S. cerevisiae*, D-4. All studies were performed both in the presence and absence of exogenous mammalian metabolic activation. Acyclovir gave no positive responses in any of these systems. The previous Salmonella studies were extended to extremely high concentrations in order to achieve toxicity. No positive effects were observed either in the presence or absence of exogenous mammalian metabolic activation, at concentrations of acyclovir up to 300mg/plate or 80mg/mL.

**Mammalian Systems:** Acyclovir was tested for mutagenic activity in cultured L5178Y mouse lymphoma cells, heterozygous at the thymidine kinase (TK) locus, by measuring the forward mutation rate to TK-deficiency (TK<sup>+/−</sup>→TK<sup>−/−</sup>); additional studies were performed at the HGPRT locus and the Ouabain-resistance marker in these same cells. All studies were
performed in the presence and in the absence of exogenous mammalian metabolic activation. The test compound was mutagenic at the TK locus at high (400-2400µg/mL) concentrations. (By comparison, acyclovir peak plasma levels following topical application are 0.27µg/mL or lower). It was negative at the HGPRT locus and Ouabain-resistance marker. Metabolic activation did not affect the results at any locus.

Inconclusive results with no apparent dose-related response were obtained when acyclovir mutagenicity was studied at each of 3 loci (APRT, HGPRT and Ouabain-resistance) in Chinese hamster ovary (CHO) cells, both in the presence and absence of exogenous metabolic activation.

Acyclovir, at a concentration of 50µg/mL (222µM) for a 72-hour exposure, has been shown to cause a statistically significant increase in the incidence of morphologically transformed foci resulting from treating BALB/C-3T3 cells in vitro in the absence of exogenous metabolic activation. The morphologically transformed foci have been shown to grow as tumors following transplantation into immunosuppressed, syngeneic, weanling mice. Tumour tissues were diagnosed as being either undifferentiated sarcomas or lymphosarcomas.

Acyclovir at concentrations between 8µg/mL and 64µg/mL for 18 hours exposure did not induce any morphologically transformed foci among C3H/10T ½ cells treated in vitro in the absence of exogenous metabolic activation.

Acyclovir, at concentrations of 62.5 and 125µg/mL for a 48-hour exposure, did not induce any chromosome aberrations in cultured human lymphocytes in the absence of exogenous metabolic activation. At higher and toxic concentrations (250 and 500µg/mL for 48 hours exposure) acyclovir caused a significant increase in the incidence of chromosome breakage.

Reproduction Studies

Largely reversible adverse effects on spermatogenesis in association with overall toxicity in rats and dogs have been reported only at systemic doses of aciclovir greatly in excess of those employed therapeutically. Two-generation studies in mice did not reveal any effect of orally administered aciclovir on fertility.
Immunotoxicology Studies

Acyclovir was subjected to a number of *in vitro* and *in vivo* immunological tests.

In two *in vitro* tests, lymphocyte-mediated cytotoxicity and neutrophil chemotaxis, acyclovir showed no inhibitory effects at concentrations as high as 135µg/mL (600µM). The compound inhibited rosette formation approximately 50% at 0.9µg/mL (4µM).

In four *in vivo* tests in mice which measured cell-mediated immunity (complement-dependent cellular cytotoxicity, complement-independent cellular cytotoxicity, delayed hypersensitivity and graft vs. host reaction) acyclovir showed no inhibitory effects at single doses up to 200mg/kg given on day 2 after antigenic stimulation.

Four daily doses of 100mg/kg/day had no significant effect on Jerne hemolysin plaques or circulating antibody on day 7 after antigenic stimulation. When the Jerne hemolysin plaques and antibody titers were examined four days after antigenic challenge and one day after the last drug dose, 100mg/kg showed only a slight suppressive effect. However, 200mg/kg produced some weight loss (-2.2g), a moderate reduction in the number of Jerne hemolysin plaques (PFC/spleen were reduced to 33% of control, PFC/10⁷ WBC to 46.5% of control). However, there was only a small reduction in the circulating hemagglutinin titer (from 8.3 to 6.5) and the circulating hemolysin titer (from 9.5 to 8.3) at 200mg/kg.

In experiments in mice designed to test whether acyclovir would potentiate the immunosuppressive effect of azathioprine on antibody formation, it was found that the effects of the two drugs were no more than additive. Only the 200mg/kg dose of acyclovir showed an increased suppression of antibody response when given in combination with azathioprine at doses above 25mg/kg.

Studies were carried out to evaluate the influence of acyclovir *in vitro* on human lymphocyte function. Inhibitory effects on blastogenesis were seen only in assays examining peak concentrations of potent mitogens, PHA and Con A, and only at concentrations of drug above 50µg/mL (222µM) and were much less with monilia and tetanus toxoid antigens, where the blastogenic response is characteristically less vigorous. There was very
little effect on cytotoxicity or LIF production except at concentrations of 200µg/mL (890µM) for which a direct cytotoxic effect has been demonstrated before. These inhibitory concentrations are far in excess of anticipated levels from doses selected for clinical application and over 1000-fold higher than the concentration required to inhibit herpesvirus multiplication \textit{in vitro}.

The effect of acyclovir on human cells was measured. A concentration of 11.2- 22.5µg/mL (50-100µM) inhibits the division of fibroblasts to a variable extent, depending on the experimental design and the confluency of the monolayer. The magnitude of this effect was less than that caused by adenine arabinoside or human leukocyte interferon when these three antiviral agents were compared at clinically relevant concentrations. Acyclovir also inhibited thymidine incorporation by peripheral blood mononuclear cells stimulated by phytohemagglutinin or three different herpesvirus antigens. A linear dose-response curve was observed with these cells, and their proliferation was 50% inhibited by 22.5µg/mL (100µM) acyclovir. Inhibition was exerted on T-cell proliferation without apparent effect on the release of lymphokines or on monocyte function.
References


