PRODUCT MONOGRAPH

[Pr]APO-ACYCLOVIR OINTMENT

Acyclovir Ointment

5% w/w

USP

Antiviral Agent

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Acyclovir Ointment, USP

5% w/w

SUMMARY PRODUCT INFORMATION

Route of Administration	Dosage Form / Strength	Non-medicinal Ingredients
Topical	Ointment 5% w/w	Polyethylene Glycol 300, Polyethylene Glycol 540

ACTION AND CLINICAL PHARMACOLOGY

APO-ACYCLOVIR OINTMENT (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

APO-ACYCLOVIR OINTMENT (acyclovir 5 %) ointment is for the management of initial episodes of genital herpes simplex infections. It is also indicated in the management of non-life-threatening cutaneous herpes simplex virus infections in immunocompromised patients. 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.

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.

These indications are based on the results of a number of double-blind, placebo-controlled studies which examined changes in virus excretion, healing of lesions and relief of pain. Because of the wide biological variations inherent in herpes simplex infections, the following summary is presented merely to illustrate the spectrum of responses observed to date. As in the treatment of any infectious disease, the best response may be expected when therapy is begun at the earliest possible moment.

The indication regarding the management of nonlife-threatening cutaneous herpes simplex virus infections in immunocompromised patients found that in immunocompromised patients, 93% were virus negative after 5 days of topical acyclovir ointment therapy, whereas only 35% of placebo recipients were virus negative at the same time. In patients with herpes labialis, there was a significantly greater decrease in the amount of virus excreted after one day of therapy in those receiving acyclovir ointment within 8 hours of the onset of cold sores when compared to identically treated placebo recipients.

Because complete re-epithelialization of herpes-disrupted integument necessitates recruitment of several complex repair mechanisms, the physician should be aware that the disappearance of visible lesions is somewhat variable and will occur later than the cessation of virus shedding. All immunocompromised patients who received topical acyclovir ointment had healed their lesions 23 days after the initiation of a 10-day course of therapy; 75% of placebo patients had healed lesions at that point. Some placebo patients continued to have visible lesions for more than 30 days.

Pain associated with herpes infections is highly variable in frequency and intensity. One hundred percent of the acyclovir ointment treated immunocompromised patients were pain-free by day 23 versus 70% of placebo-treated patients.

CONTRAINDICATIONS

APO-ACYCLOVIR OINTMENT is contraindicated for patients who develop hypersensitivity or chemical intolerance to acyclovir, valacyclovir or any of the components of the formulation, such as polyethylene glycol.

WARNINGS

APO-ACYCLOVIR OINTMENT is intended for topical use only and should not be used in the eye or on mucous membranes, such as the mouth or vagina.

PRECAUTIONS

General

The recommended dosage, frequency of application and duration of treatment of APO-ACYCLOVIR OINTMENT should not be exceeded (see DOSAGE AND ADMINISTRATION).

There exist no data, at this time, which demonstrate that the use of acyclovir ointment 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 APO-ACYCLOVIR OINTMENT will prevent recurrence of infections when applied in the absence of signs and symptoms. APO-ACYCLOVIR OINTMENT 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 APO-ACYCLOVIR OINTMENT 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 1 g 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 APO-ACYCLOVIR OINTMENT and although evidence suggests that absorption of acyclovir through the skin is minimal, caution should be exercised when acyclovir is administered to a nursing mother.

Use in Pregnancy

APO-ACYCLOVIR OINTMENT should not be used during pregnancy unless the physician feels the potential benefit justifies the risk of possible harm to the fetus.

Teratology studies using acyclovir cream 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; and while since such studies are not always predictive of human response, the potential for high concentrations of acyclovir to cause chromosome breaks *in vitro* should be taken into consideration in making this decision.

All animal studies with the ointment carried out to date on reproduction and teratology have been negative. However, animal reproduction studies are not always predictive of human response.

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

Pediatrics Patients (< 18 years of age)

The safe use of acyclovir in pediatric patients less than 18 years of age has not been established

DRUG INTERACTIONS

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

ADVERSE REACTIONS

Because ulcerated genital lesions are characteristically tender and sensitive to any contact or manipulation, patients may experience discomfort upon application of acyclovir.

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

Ointment

In the controlled clinical trials, mild pain (including transient burning and stinging) was reported by 103 (28.3%) of 364 patients treated with acyclovir ointment and by 115 (31.1%) of 370 patients treated with placebo; treatment was discontinued in 2 of these patients. Other local reactions among acyclovir-treated patients included pruritus in 15 (4.1%), rash in 1 (0.3%) and vulvitis in 1 (0.3%). Among the placebo-treated patients, pruritus was reported by 17 (4.6%) and rash by 1 (0.3%).

In all studies, there was no significant difference between the drug and placebo group in the rate or type of reported adverse reactions.

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 APO-ACYCLOVIR OINTMENT is unlikely because of limited transcutaneous absorption.

For management of a suspected drug overdose, contact your regional poison control centre.

DOSAGE AND ADMINISTRATION

Apply APO-ACYCLOVIR OINTMENT 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 APO-ACYCLOVIR OINTMENT in order to prevent: (1) autoinoculation of other body sites or (2) transmission of infection to other persons. **Therapy should be initiated as early as possible following onset of signs and symptoms.**

PHARMACEUTICAL INFORMATION

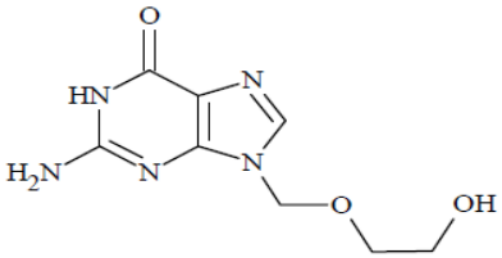
Drug Substance

Proper Name: Acyclovir

Chemical Name: 9-[(2-hydroxyethoxymethyl)] guanine

Other Names: Acycloguanosine

Structural Formula:



Molecular Formula: $C_8H_{11}N_5O_3$

Molecular Weight: 225.2 g/mol.

Physicochemical Properties

Description: Acyclovir is white or almost white, crystalline powder.

Solubility: Slightly soluble in water, very slightly soluble in ethanol (96%), practically insoluble in heptane. It dissolved in dilute solutions of mineral acids and alkali hydroxides.

AVAILABILITY OF DOSAGE FORMS

Each gram contains 50 mg acyclovir in a polyethylene glycol base.

APO-ACYCLOVIR OINTMENT is a white to off-white smooth homogenous ointment, free from lumps and foreign matter and without phase separation supplied in collapsible aluminium tube with white FEZ cap with puncture tip. APO-ACYCLOVIR OINTMENT is available in tubes of 4 g, 5 g, 15 g and 30 g.

STORAGE CONDITIONS

Store 15ºC- 25ºC. Keep in a dry place.

VIROLOGY

The quantitative relationship between the *in vitro* susceptibility of herpes simplex virus (HSV), varicella-zoster virus (VZV) and cytomegalovirus to acyclovir and the clinical response to therapy has not been established in man, and virus sensitivity testing has not been standardized.

Prolonged exposure of herpes simplex virus (HSV) to subinhibitory concentrations (0.1 mcg/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 *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 *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 *in vitro* virus sensitivity and clinical response to acyclovir therapy.

Ointment

In Vitro Activity

The techniques and cell culture types used for determining *in vitro* susceptibility may influence the results obtained. Using a quantitative assay to determine the acyclovir concentration producing 50% inhibition of viral cytopathic effect (ID_{50}), 28 HSV-1 clinical isolates had an ID_{50} range of 0.02 to 0.70 mcg/mL (mean - 0.17 mcg/mL), and 32 HSV-2 clinical isolates had an ID_{50} range of 0.01 to 3.16 mcg/mL (mean - 0.46 mcg/mL).[23] Results from other studies using different assays have yielded mean ID_{50} values for clinical HSV-1 isolates of 0.018, 0.03 and 0.043 mcg/mL and for clinical HSV-2 isolates of 0.027, 0.36 and 0.03 mcg/mL, respectively.[2,3,4]

Using a plaque reduction assay, 9 clinical isolates of VZV had an ID_{50} range of 0.70 to 2.32 mcg/mL (mean - 1.52 mcg/mL).

A reduced spectrum of *in vitro* activity was evident with 5 clinical isolates of CMV, where again using a plaque reduction assay an ID_{50} range was found of 2.25 to 12.81 mcg/mL (mean - 8.22 mcg/mL).

Enzyme Studies - Effects of Acyclovir-TP on Viral and Cellular DNA Polymerases

Time of addition studies with acyclovir showed that the compound was effective early in the replicative cycle of HSV-1, i.e., prior to 8 hours post-infection. If the compound was added to HSV-infected Vero cells on monolayers after 8 hours post-infection, no antiviral activity was observed.2 The formation of acyclovir-TP was detectable at 2 hours and reached a maximum 8 hours after drug addition of HSV-infected cultures. These results suggested that acyclovir was activated and exerted its inhibitory effect at some point early in the replicative cycle of HSV-1, most likely during DNA synthesis.

The HSV-specified DNA polymerase has been identified as one of the major enzymes appearing early after infection. It, therefore, seemed a probable target enzyme for the action of acyclovir-TP. Herpes-specified DNA polymerase was purified from HeLa cells infected with HSV-1, using DEAE column chromatography followed by phosphocellulose column chromatography. The cellular DNA polymerase-α from these infected cells was likewise isolated. Kinetic studies were then performed with various synthetic templates (dC.odG and dA.odT) to verify the identities of the viral and cellular DNA polymerases. The inhibitory effect of acyclovir-TP on these two DNA polymerases was then determined. The results obtained showed that acyclovir-TP was a competitive inhibitor of dGTP for both the viral and cellular DNA polymerases. However, in all cases, acyclovir-TP was a better inhibitor of the viral DNA polymerase than of the cellular DNA polymerase. Inhibition constants have been obtained with the isolated viral DNA polymerases of HSV-1 (strains H29, KOS, and MacIntyre), HSV-2 (strains MS and 333), and human cytomegalovirus (strain AD 169), as well as of the cellular α-DNA polymerases of HeLa S-3, Vero, WI-38 and L-929 cells. These are listed below.

Source of Virus-Induced or α-cellular DNA Polymerase	**Apparent Ki (mcM acyclo-GTP**
HSV-1 (strain H29)	0.08
HSV-1 (strain MacIntyre)	1.42
HSV-1 (strain KOS)	0.55
HSV-2 (strain MS)	0.45
HSV-2 (strain 333)	0.56
Human cytmegalovirus (strain AD169)	0.25
HeLa S-3	2.34
Vero	2.09
WI-38	1.86
L-929	2.80

Thus, one locus of action for acyclovir-TP appears to be inhibited of viral DNA polymerase. Since this inhibition is greater for the viral enzyme than for the cellular enzyme, the selectivity of acyclovir is further increased. This, couples with the much larger amount of acyclovir-TP present

in herpesvirus-infected cells, helps to explain the large difference in the ability of the drug to inhibit herpesvirus (strain H29)[5] multiplication (ED_{50}=0.1 mcM=22.5 mcg/mL) compared with its inhibition of Vero cell multiplication (ED_{50}=300 mcM=67.5 mcg/mL).

There is evidence from enzyme studies with viral DNA polymerase that acyclovir is incorporated into viral DNA. This incorporation is rapid at first, if one uses 25 mcM acyclovir triphosphate, but quickly comes to a stop due to inactivation of the polymerase[6]. Incorporation of acyclovir into DNA would be expected to be a chain-terminating event, since there is no hydroxyl group corresponding to the 3'-OH group of deoxyribose to form a 3',5'-phosphodiester linkage. Such a chain-termination has now been demonstrated in the transformed cell line LH7, which was transformed from TK^- or TK^+ by a DNA fragment of HSV-1. This cell line expresses the HSV-TK, makes acyclovir triphosphate and incorporates acyclovir into DNA chains which are terminated by such incorporation.

Challenge Studies

Challenge studies have been carried out in a wide variety of animals, such as mice, rabbits, guinea pigs and monkeys. Acyclovir produced rapid healing of rabbit eye keratitis when administered as a 3% ointment (using the contralateral eye as a control). It was as effective as iododeoxyuridine, trifluorothymidine or superior to trifluorothymidine, vidaravine and idoxuridine. Both the topical and intravenous forms of acyclovir have offered partial to total protection, respectively, from a fatal outcome due to encephalitis following inoculation of herpes simplex into animal eyes.

A therapeutic effect has been seen in mouse herpes encephalitis using oral doses of 100 mg/kg twice daily for 5 days,14 or with continuous oral doses of 400 mg/kg for 7 days.

It has also been shown that subcutaneous dosing of 100 mg/kg/day initiated 12 hours after viral inoculation for 4 consecutive days affects the survival rates and vital titers in the mouse herpes simplex encephalitis model.

Topical therapy has been shown to be effective in mice against herpes simplex virus skin lesions. Similar results were shown in guinea pigs.

Intravenous acyclovir has also been shown to be effective in experimental herpes B virus infection in the rabbit.

Resistance

A colorimetric method has been used to quantitate the inhibition of viral cytopathic effect. From the data, one can calculate the drug concentration producing a 50% inhibition of viral replication (ID_{50}) (this assay gives ID_{50} values which are approximately ten times higher than ID_{50}'s obtained in a plaque reduction assay). McLaren et al. reported a study in which ID_{50}'s of clinical isolates from patients with herpes simplex type 1 or type 2 infections were measured in the CPE-reduction assay. The mean ID_{50} value for the HSV-1 isolates was 0.17 mcg/mL while the mean of the HSV-2 isolates was somewhat higher at 0.46 mcg/mL. Based on initial results, virus

isolates with ID_{50}'s greater than 2.0 mcg/mL are tentatively regarded as having a significantly lower sensitivity to acyclovir.

It is possible to isolate strains of HSV which show a reduced sensitivity to the drug, by growing the virus in tissue cultures treated with acyclovir. Such 'resistant' viruses are generally deficient in thymidine kinase activity (TK). A few mutants with changed DNA-polymerase have also been isolated in laboratory experiments but with a much lower frequency. Experiments in animals have indicated that although the TK-deficient mutants of HSV are capable of infecting and inducing antibody production, they generally have a greatly reduced ability to cause disease and have a significantly diminished ability to establish latent infections in neuronal ganglia. The clinical significance of these findings has yet to be determined. To date, no consistent correlation has been established between resistance identified in the laboratory and clinical expression of HSV disease.

Virus with a diminished sensitivity to acyclovir (an increased ID_{50}) has been detected in seven patients treated with IV acyclovir and three patients treated with the topical ointment. These particular virus isolates also showed reduced thymidine kinase activity.

The majority of the patients treated with intravenous drug from whom less sensitive virus was isolated were severely immunocompromised by virtue of chemotherapy or innate deficiencies. In two bone-marrow transplant patients TK-deficient HSV was shed asymptomatically for a brief period after a significant clinical response to intravenous acyclovir therapy. Viruses with reduced TK-activity were also isolated from two children with severe immunodeficiencies following successful initial therapy with acyclovir; the clinical disease in these children neither improved nor worsened following the emergence of TK-deficient virus. In the patients who survived their underlying disease, TK-deficient virus has not been detected during subsequent recurrences nor has any such isolate apparently spread nosocomially. In immunocompetent patients with genital HSV-2 infections treated with acyclovir ointment, cessation of virus replication and lesion healing occurred in a time period similar to that in other patients who had viruses with normal TK activity.

Among the placebo-treated patients, a change in viral sensitivity was found in two patients treated with the topical preparation. In only one of these patients was the increase in ID_{50} associated with diminished TK activity. Five patients given placebo intravenously exhibited changes in the *in vitro* sensitivity of their virus populations during or after administration of the placebo. The TK activity was reduced in two, remained unchanged in one and was not tested in three of these patients.

A number of patients have been identified in which the virus presents prior to therapy already exhibited a relatively high ID_{50}, sometimes associated with low TK activity. In the intravenous studies, there were six such patients with less sensitive virus; virus with a similar sensitivity was also recovered from the cervix, but not from other lesions, of a seventh patient.

PHARMACOLOGY

Ointment

Acyclovir administration to adults at 5 mg/kg (approximately 250 mg/m^2 BSA) by 1-hour infusions every 8 hours produces mean steady-state peak and trough concentrations of 9.8 mcg/mL and 0.7 mcg/mL, respectively.

Similar concentrations are achieved in pediatric patients over 1 year of age when doses of 250 mg/m^2 BSA (body surface area) are given every 8 hours. Concentrations achieved in the cerebrospinal fluid are approximately 50% of plasma values. Plasma protein binding is relatively low (9 to 33%) and drug interactions involving binding site displacement are not anticipated. Renal excretion of unchanged drug by glomerular filtration and tubular secretion is the major route of acyclovir elimination accounting for 62 to 91% of the dose in man as determined by ^{14}C-labelled drug. The only significant urinary metabolite is 9-carboxymethoxymethylguanine. An insignificant amount of drug is recovered in feces and expired CO_2 and there is no evidence to suggest tissue retention.

The half-life and total body clearance of acyclovir is dependent on renal function as shown below.

Creatinine Clearance (mL/min/1.73 m^2 BSA*)	**Half-Life (hr)**	**Total Body Clearance (mL/min/1.73 m^2 BSA*)**
>80	2.4	33225118526
50-80	2.9	
15-50	3.7	
0 (Anuric)	18	

* Body surface area

The half-life and total body clearance of acyclovir in pediatric patients over 1 year of age is similar to adults with normal renal function.

Two clinical pharmacology studies were performed with acyclovir ointment in adult immunocompromised patients, at risk of developing mucocutaneous herpes simplex virus infections or with localized varicella-zoster infections. These studies were designed to evaluate the dermal tolerance, systemic toxicity and percutaneous absorption of acyclovir. In one of these studies, which included 16 inpatients, the complete ointment or its vehicle were randomly administered in a dose of 1cm strips (25 mg acyclovir) four times a day for seven days to an intact skin surface area of 4.5 square inches. No local intolerance, systemic toxicity or contact dermatitis were observed. In addition, no drug was detected in blood and urine by radioimmunoassay[30] (sensitivity, 0.01 mcg/mL).

The other study included 11 patients with localized varicella-zoster. In this uncontrolled study, acyclovir was detected in the blood of 9 patients and in the urine of all patients tested. Acyclovir levels in plasma ranged from 0.01 to 0.28 mcg/mL in eight patients with normal renal function, and from 0.01 to 0.78 mcg/mL in one patient with impaired renal function. Acyclovir excreted in the urine ranged from 0.02 to 53.6 mcg/mL (0.02 to 9.4%) of the daily dose. Therefore, systemic absorption of acyclovir after topical application is minimal.

TOXICOLOGY

Acute Toxicity Studies

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

Species	Sex	Route	LD_{50} (mg/kg)	95% Conf. Level	Signs
Mouse	M	Oral	>10000	-	None
Rat	M	Oral	>20000	-	None
Mouse	M	IV	405	-	Ataxia Depression
Rat	M	IV	>600	-	None
Mouse	M	IP	1454	1323- 1662	Sedation
Mouse	F	IP	999	670- 1364	Sedation
Rat	M	IP	1305	512-1733	Sedation
Rat	F	IP	1210	504- 1580	Sedation
Rabbit	M/F	Dermal	>2 (g/kg)	-	None

Subchronic Oral Toxicity Study

Mice
Four groups each consisting of 28 male and 28 female Charles River CD-1 (ICR) mice were orally dosed by stomach tube for 33 days with suspensions of acyclovir. Daily dose levels were 0, 50, 150 and 450 mg/kg. Hematology and clinical chemistry measurements were made on an additional 8 male and 8 female mice per group (dosed in the same manner) after the first and fourth weeks of dosing and during the 3rd postdose week.

Plasma drug concentrations were measured in pooled samples from an additional 4 male and 4 female mice per group on dose days 1, 15 and 31.

Based on preliminary experiments with rats and mice, the high dose of 450 mg/kg was selected to produce the highest drug plasma levels attainable, in a practical manner, by oral dosing in a rodent species. Averaged drug plasma concentrations ranged from approximately 3.4 (at the low dose) to 11.0 (at the high dose) mcg/mL of plasma one hour after oral dosing.

No changes in health, growth rate, hematology and clinical chemistry measurements occurred that could be definitely attributed to dosing with acyclovir. Gross and histopathologic examinations of 16 male and 16 female rats from the high-dose and control groups at the end of the dose period revealed nothing remarkable.

Subchronic Intravenous Toxicity Studies

Beagle Dogs
In a 31-day study in Beagle dogs, acyclovir was administered as a bolus intravenous injection to groups of 8 dogs (4 male and 4 female) at dosage levels of 0, 25, 50 and 100 mg/kg, b.i.d.

Intravenous bolus doses of 50 or 100 mg/kg, b.i.d. per day in this study produced very high drug plasma levels [mean values in the range of 45 to 254 mcg/mL (200 to 1127 mcM)] which were obviously highly toxic, whereas the 25 mg/kg, b.i.d. dose resulted in considerably lower plasma levels in the range of 22.5 to 45 mcg/mL (100 to 200 mcM) and was only marginally toxic and nearly a "no effect" dose.

Primary drug-related changes at the 25 mg/kg b.i.d. dose level included: infrequent retching and/or emesis, occasional tachycardia, increased urinary output with a decrease in specific gravity. These effects were reversible and undetectable 15 days after withdrawal of treatment. At the 50 and 100 mg/kg b.i.d. dosage, additional adverse effects were seen including dyspnea, hypothermia, hypoactivity, bloody or mucoid diarrhea, dehydration, body weight loss, partial to total anorexia, leucopenia, slight increases in serum total protein, albumin, creatinine, urea nitrogen and occasional "loud" heartbeat.

Findings considered directly related to drug treatment with the 50 and 100 mg/kg, b.i.d. dosage levels included infrequent retching and emesis, occasional tachycardia and "loud" heartbeat, increased urine output, hyaline droplets in the cytoplasm of the liver parenchymal cells, mild cytologic changes in the colon mucosa and kidney toxicity. Some other changes, considered secondary to the effects of drug administration at the 50 and 100 mg/kg b.i.d. levels, were skeletal muscle and adipose tissue atrophy, depletion of lipid from cortex of adrenal gland, and aspermic testes.

More seriously, there were tremors, cyanosis, prostration and early death (within the first 8 days of the study).

Rats
Groups of 15 male and 15 female Sprague-Dawley rats were given one daily intravenous injection as a bolus of 20, 40 or 80 mg/kg of acyclovir for 20 or 21 consecutive days. Drug was formulated as a 2 % isotonic solution in sterile 0.4 % sodium chloride. A control group was given single daily injections of a 0.9% solution of sodium chloride.

At all dose levels most or all rats had renal lesions which were considered to be related to obstruction of the distal nephron by precipitated drug crystals. Lesions increased in severity with rising dose and were those to be expected with obstruction of the distal nephron: retrograde tubular dilatation and epithelial degeneration, necrosis and regeneration. There was an accompanying interstitial inflammatory component in some of the more severely affected kidneys.

Birefringent drug crystals were visible in sections of kidney which had been frozen before formalin fixation but were not seen in conventional paraffin sections since they had been solubilized by the processes of formalin fixation and staining.

Examination of kidney sections from rats which had been maintained for a 15-day drug-free period after administration of the last dose revealed only mild residual reparative changes or no lesions at all. This indicates the reversibility of the obstructive nephropathy.

Clinical effects of drug were considered to be related to renal changes. These consisted of reduced body weight, elevated blood urea nitrogen values, increased water intake and urine output plus an increase in mean absolute and relative kidney weights.

In a second study in rats, lower drug doses were used in an effort to establish a no-effect level. The doses were 5 or 10 mg/kg/day given as a single bolus intravenous injection for 19 to 20 consecutive days, and there was a control group which received 0.9% sodium chloride solution. The only finding in the rats, which was considered definitely associated with drug administration, was very mild dilatation of distal tubules in kidneys of 2 of 20 animals in the 5 mg/kg group. The dilatation was thought to be due to recent, and perhaps still present, distal nephron obstruction by drug crystals (though they were not present in paraffin sections for the reason explained above).

Long-Term Toxicity Studies

12-Month Toxicity Study in Dogs

Purebred Beagle dogs were given 0, 15, 45 or 150 mg/kg/day of acyclovir each day for the first two weeks of a 1-year study. There were 9 male and 9 female dogs in each test group. The dogs were given gelatin capsules that contained the appropriate dose. They were treated t.i.d., hence the dosages administered at each of three equally spaced dose periods were 0, 5, 15 and 50 mg/kg. The 45 and 150 mg/kg dose levels induced diarrhea, emesis, decreased food consumption and weight loss in both male and female dogs during the first two weeks of the study. For this reason, during the third week of the study the decision was made to decrease the mid and high dosage levels to 30 and 60 mg/kg/day (10 and 20 mg/kg t.i.d.). The low dose of 15 mg/kg/day (5 mg/kg t.i.d.) was unchanged. Dogs given 60 mg/kg/day occasionally vomited and occasionally had diarrhea but did well for the duration of the test and values for body weight gain and food consumption were comparable to control values.

During the toxicosis induced by the larger doses of acyclovir, plasma levels of the drug were likely very high (as indicated by initial mean values of 24.0 mcg/mL (106.6 mcM) for high-dose males and 17.4 mcg/mL (77.2 mcM) for high-dose females when determined 1 hour after the third dose on day 1 of the study). When measured on day 15, plasma levels of acyclovir in high-dose dogs (150 mg/kg/day) were still very high but they decreased later when the dosages were decreased. Values for plasma levels after 12 months of treatment were generally comparable to values recorded after 1, 3 and 6 months of treatment. Thus, there was no indication of enhanced metabolism of acyclovir as a result of chronic treatment.

During the 13th week, some male and female dogs at both the mid and high dosage levels had the following signs: tenderness in forepaws, breaking of nails and loosening of nails. Regeneration of lost nails began a few weeks later. Nails regenerated by 6 months (when 3 males and 3 females from each group were killed for an interim sacrifice) and by the end of the study

were of generally good quality. There were never any signs of an effect on paws or nails in dogs in the low-dose group (15 mg/kg/day).

It is accepted that injury of the corial epithelium that produces nail keratin can result in arrested production of keratin and production of abnormal keratin. The transient toxicosis induced by the large doses (45 and 150 mg/kg/day) of acyclovir given during the first two weeks of the study may have affected the corial epithelium. If there was a transient effect on the corial epithelium (possibly related to direct effects or secondary to drug-induced illness during the first two weeks of the study) later loss of the nail could be a sequella. No discernible effects upon other keratin-producing or keratin-containing tissues were observed. It should be emphasized that the alterations in the nails appeared to be related to the transient toxicosis induced by dose levels of 50 and 150 mg/kg/day tested during the first two weeks of the study and not to the 30 and 60 mg/kg/day dose levels tested subsequently.

There were no important drug-induced alterations in values for serum biochemical tests, urinalyses and electrocardiographic tests done at appropriate intervals during this study. Values for serum albumin and total protein were slightly decreased in dogs treated at 30 and 60 mg/kg/day for 6 and 12 months. However, all values for these parameters remained within limits accepted as normal.

With the exception of residual alterations in old keratin at the tips of the claws, there were no signs of treatment-related effects in any of the tissues examined by light microscopy. Nor were there meaningful alterations in values for the organs weighed at necropsy. Thus, dose levels up to 60 mg/kg/day were well tolerated. Except for mild gastrointestinal signs at 60 mg/kg/day, all dose levels tested for 1 year were “no effect” levels.

52-Week Interim Report of 104-Week Oral Toxicity Study in Rats Given Acyclovir by Gastric Intubation

Charles River CD (Sprague-Dawley) rats were given suspensions of acyclovir by gavage for 52 weeks of a 104-week study. There were 50 male and 50 female rats at each of the following dose levels: 0, 50, 150 and 450 mg/kg. After 30 and 52 weeks of treatment, 10 male and 10 female rats from each group were necropsied. Tissues from control rats and those in the high dose group were evaluated by light microscopy for both the 30- and 52-week interim sacrifices. Fixed tissues from rats that were found dead during the first 52 weeks of the study were also evaluated by light microscopy.

No signs of toxicosis were observed. Mean plasma levels were obtained in high-dose males (450 mg/kg/day) 1.5 hours after dosing at various sampling times during the study as follows: 1.54, 1.63, 1.39 and 1.60 mcg/mL (6.84, 7.26, 6.17 and 7.10 mcM) at days 7, 90, 209 and 365, respectively.

Corresponding mean values for the high-dose females were 1.76, 2.38, 2.12 and 1.71 mcg/mL (7.82, 10.58, 9.44 and 7.62 mcM). Plasma levels in both males and females at all dose levels after one year of treatment were generally comparable to plasma levels obtained at earlier samplings. Values for laboratory tests including hematology, clinical chemistry and ophthalmoscopy were all within the normal range. There were no drug-induced gross or

microscopic lesions in rats killed for 30- and 52-week interim sacrifices. Most of the relatively few rats found dead or moribund during the first 52 weeks of this study suffered dosing accidents as evidenced by postmortem findings of esophageal perforation causing pleural effusion, pneumonia, or mediastinitis.

Genotoxicity and Mutagenicity 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/1OT 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 (100 mg/kg) in rats or Chinese hamsters; at higher doses (500 and 1000 mg/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 300 mg/plate or 80 mg/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 ($TK^{+/-} \rightarrow TK^{-/-}$); 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 to 2400 mcg/mL) concentrations. (By comparison, acyclovir peak plasma levels following topical application are 0.27 mcg/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 50 mcg/mL (222 mcM) 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. Tumor tissues were diagnosed as being either undifferentiated sarcomas or lymphosarcomas.

Acyclovir at concentrations between 8 mcg/mL and 64 mcg/mL for 18 hours exposure did not induce any morphologically transformed foci among C_3H_{10} T½ cells treated *in vitro* in the absence of exogenous metabolic activation.

Acyclovir, at concentrations of 62.5 and 125 mcg/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 500 mcg/mL for 48 hours exposure) acyclovir caused a significant increase in the incidence of chromosome breakage.

Reproduction/Fertility Study
Acyclovir, at single intraperitoneal doses of 25, 50 and 100 mg/kg, failed to induce chromosome aberrations in bone marrow cells of Chinese hamsters when examined 24 hours after dosing. At higher doses (500 and 1000 mg/kg), a clastogenic effect was seen. (An intraperitoneal dose of 500 mg/kg produces mean peak plasma levels in Chinese hamsters of 611 mcg/mL (2.72 mM) which is 2200 times higher than human plasma levels following recommended topical application).

Acyclovir, at single intravenous doses of 25, 50 and 100 mg/kg, failed to induce chromosome aberrations in bone marrow cells of male and female rats when examined at 6, 24 and 48 hours after treatment.

Carcinogenicity Studies

Lifetime Oral Carcinogenicity Study in Rats
There were no signs of toxicosis in Charles River CD (Sprague-Dawley) rats (100 rats/sex/dose group) given acyclovir by oral gavage at 50, 150 and 450 mg/kg in a lifetime oral carcinogenicity study. Mean plasma levels obtained in high-dose males 1.5 hours after dosing at various sampling times during the study were as follows: 1.54, 1.63, 1.39, 1.60 and 1.70 mcg/mL (6.84, 7.26, 6.17, 7.10 and 7.56 mcM) at days 7, 90, 209, 369 and 771, respectively.

Corresponding mean values for the high-dose females were 1.76, 2.38, 2.12, 1.71 and 1.81 mcg/mL (7.82, 10.58, 9.44, 7.62 and 8.03 mcM).

Values for clinical laboratory tests including hematology, clinical chemistry, urinalysis, body weight, food consumption and ophthalmoscopy were all within normal ranges. There were no drug-induced gross or microscopic lesions and there was no evidence that acyclovir affected survival, temporal patterns of tumor incidence or tumor counts for benign or malignant neoplasms.

Lifetime Oral Carcinogenicity Study in Mice
There were no signs of toxicosis in Charles River CD-1 (ICR) mice (115 mice/sex/dose group) given acyclovir by oral gavage at 50, 150 and 450 mg/kg/day in a lifetime oral carcinogenicity study. Mean plasma levels obtained in high-dose males 1.5 hours after dosing at various sampling times during the study were as follows: 2.83, 3.17 and 1.82 mcg/mL (12.59, 14.10 and 8.10 mcM) at days 90, 365 and 541, respectively. Corresponding mean values for the high-dose females were 9.81, 5.85 and 4.0 mcg/mL (43.60, 26.0 and 17.79 mcM).

Values for clinical laboratory tests including hematology, body weight and food consumption were all within normal ranges. There were no drug-induced gross or microscopic lesions. Female mice given 150 and 450 mg/kg acyclovir survived significantly longer than control female mice; survival of treated males was comparable to survival of control males. Patterns of tumor incidence and tumor counts for benign or malignant neoplasms were not affected by treatment with acyclovir.

Reproduction Studies

Teratology - Rats
Acyclovir was administered to pregnant A.R.S. Sprague-Dawley female rats by subcutaneous injection during the period of organogenesis (day 6 through day 15 of gestation) at dose levels of 0.0, 6.0, 12.5 and 25.0 mg/kg body weight twice daily.

Criteria evaluated for compound effect included maternal body weights, weight gains, appearance and behavior, survival rates, eye changes, pregnancy rates, and reproduction data. Offspring viability and development were also evaluated.

In addition to the above measurements, designated animals were sacrificed 1 hour after the first dose on day 15 in order to collect samples of maternal blood, amniotic fluid and fetuses for measurements of drug concentration. Mean values from these samples were as follows:

Acyclovir Concentrations			
Dose mg/kg b.i.d., s.c.	**Plasma (mcg/mL)**	**Amniotic Fluid (mcg/mL)**	**Fetal Homogenate ng/g (nmoles/g w/w)**
6 N = 7	0.26 ± 0.09	0.39 ± 0.06	0.704 (3.13 ± 0.50)
12.5 N = 5	0.69 ± 0.20	1.13 ± 0.22	0.963 (4.28 ± 0.67)
25 N = 5	1.59 ± 0.55	2.0 ± 0.53	1.994 (8.64 ± 2.33)

The values obtained for plasma would represent about 30% of initial plasma levels as judged by the plasma half-life in rodents.

No effects attributable to the administration of acyclovir were noted in comparisons of maternal body weight values, appearance and behavior, survival rates, pregnancy rates, or implantation efficiencies. In addition, no compound-related differences were noted in evaluations of fetal size, sex, and development.

Although the incidences of resorption and fetal viability were within the range of normal variability in all of the groups, slightly greater incidences of resorptions were noted in the high-dose animals sacrificed on days 15 and 19 of gestation; however, clear dose-related trends did not eventuate. Therefore, acyclovir was not considered teratogenic or embryotoxic when administered to rats at levels up to 50.0 mg/kg of body weight per day during organogenesis.

Teratology - Rabbits
A teratology study was done in New Zealand White rabbits using essentially the same experimental design as in the rat, except that dosing was from day 6 through day 18 of gestation. Also, collection of fetuses, amniotic fluid and samples of maternal blood occurred on day 18 rather than day 15.

No signs of maternal toxicity were observed at any dose, but there was a statistically significant ($p<0.05$) lower implantation efficiency in the high-dose group. While there were a few terata observed in the study (in both control and treated animals), there was no apparent association with drug treatment. There was, however, an apparent dose-related response in the number of fetuses having supernumerary ribs. No similar effect was noted in the rat teratology study (see above) or in a reproduction-fertility experiment in mice.

Concentrations of acyclovir were detected in plasma and amniotic fluid samples, as well as in homogenates of fetal tissues. All samples were taken one hour after the first dose on day 18 of gestation. As apparent in the following data, drug concentrations in amniotic fluid were substantially higher than that of plasma.

Acyclovir Concentrations (Mean and S.E.)			
Dose mg/kg b.i.d., s.c.	**Plasma (mcg/mL)**	**Amniotic Fluid (mcg/mL)**	**Fetal Homogenate ng/g (nmoles/g w/w)**
6 N = 4	0.25 ± 0.03	0.89 ± 0.18	0.155 (0.69 ± 0.13)
12.5 N = 5	0.25 ± 0.05	8.03 ± 6.37	0.207 (0.92 ± 0.14)
25 N = 5	1.39 ± 0.12*	6.16 ± 4.25	0.315 (1.40 ± 0.19)
*N = 5			

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

Developmental Toxicity Studies

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 LD_{50} 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.

	LD_{50} (mg/kg body weight)	
Age When Treated	**Males**	**Females**
3 Days	1070	1281
10 Days	790	496
28 Days	678	750
71 Days	650	1477

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.

Neonatal Rats - Subchronic Study

Acyclovir dissolved in 0.4% sterile saline was given by subcutaneous injection to Charles River CD (Sprague-Dawley) neonatal rats for 19 consecutive days, beginning on the 3rd post-partum day. The dose levels tested were 0, 5, 20 and 80 mg/kg body weight. There were 12 litters (each consisting of 5 male and 5 female neonates nursing the natural dam) at each dose level. The dams were not treated. Neonates were removed from each group for necropsy and microscopic evaluation of a wide variety of tissues, including eyes and multiple sections of brain, after they had been treated for 5, 12 or 19 days and after a 3-week postdose drug-free period (at which time they were 45 days of age). Hematologic (hemoglobin, packed cell volume, RBC, WBC and differential cell counts) and clinical chemistry (BUN) tests were done after 16 days of treatment and repeated 18 days after the last (19th) dose was given.

Blood was collected from some neonates 30 minutes after treatment on day 1, on day 9 and at the end of the dose period for the determination of concentrations of acyclovir in plasma. The largest concentration of acyclovir in plasma was 99.1 mcg/mL (440.5 mcM) found in pooled plasma collected from 6 female high-dose (80 mg/kg) neonates 30 minutes after the first dose was given. Treatment with acyclovir did not increase mortality in the neonatal period.

Rats in the low-dose group gained as much body weight as the respective control rats. Significant ($p<0.05$) reductions in mean body weight values were observed in mid- and high-dose group male and female neonates during the treatment period. Rats in the high-dose group partially

compensated by gaining significantly more body weight than the controls during the postdose recovery period. There was a minimal but significant increase in BUN for male ($p<0.01$) and female ($p<0.05$) neonates in the high-dose group on dose day 16. This finding may be of biological importance because there were minimal accumulations of nuclear debris in renal collecting ducts and loops of Henle in kidney sections taken from high-dose neonates after 19 days of treatment and examined by light microscopy. This was the only time period (and the kidney was the only organ) in which minimal effects on developing organ systems were detected. Thus, 5 mg/kg was clearly a no effect dose level and 20 mg/kg caused only minimal decreases in body weight gain.

Eye examinations and light microscopy did not reveal adverse effects on ocular development. It should be emphasized that there was no morphologic or functional evidence of adverse effects on developing brain or other portions of the central nervous system. Thus, acyclovir is distinctly different than cytosine arabinoside which was reported to produce prominent cerebellar and retinal dysplasia in neonatal rats.

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 135 mcg/mL (600 mcM). The compound inhibited rosette formation approximately 50% at 0.9 mcg/mL (4 mcM).

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 200 mg/kg given on day 2 after antigenic stimulation.

Four daily doses of 100 mg/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, 100 mg/kg showed only a slight suppressive effect. However, 200 mg/kg produced some weight loss (-2.2 g), a moderate reduction in the number of Jerne hemolysin plaques (PFC/spleen were reduced to 33% of control, PFC/10^7 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 200 mg/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 200 mg/kg dose of acyclovir showed an increased suppression of antibody response when given in combination with azathioprine at doses above 25 mg/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 50 mcg/mL (222 mcM) 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 200 mcg/mL (890 mcM) 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 *in vitro*.

The effect of acyclovir on human cells was measured. A concentration of 11.2 to 22.5 mcg/mL (50 to 100 mcM) 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 22.5 mcg/mL (100 mcM) acyclovir. Inhibition was exerted on T-cell proliferation without apparent effect on the release of lymphokines or on monocyte function.

Ointment

Dermal Irritation and Systemic Toxicity
Repeated daily dermal application (4x/day at 4-hour intervals) of 5 and 10% acyclovir in polyethylene glycol ointment base (200 mg q.i.d.) to the intact and abraded skin (10% body surface) of guinea pigs for 23 to 24 days, produced no adverse local effects. There was a decrease in mean lymphocytes (in abraded female) and in red blood cell counts (abraded male and female) in animals treated with the 10% acyclovir formulation. Mean drug plasma concentration showed a considerable variation within any given treatment group (viz: 5% ointment, abraded male, 1.02 mcg/mL (4.51 mcM), abraded female, 0.4 mcg/mL (1.78 mcM); 10% ointment, abraded male, 0.54 mcg/mL (2.41 mcM), abraded female, 1.24 mcg/mL (5.52 mcM); 10% ointment, intact male, 0.9 mcg/mL (4.00 mcM); intact female 0.81 mcg/mL (3.60 mcM). The results indicate that acyclovir applied as a PEG-base ointment does penetrate the dermal barrier and is absorbed into the systemic circulation.

Epidermal Wound Healing
Spruance and Krueger tested a 5% acyclovir ointment in polyethylene glycol vehicle for potential effects on epidermal wound healing in domestic pigs. They used a dermatome to make 0.5 to 1.0 cm^2 x 0.3 mm deep wounds on the backs of the pigs. The wounds were excised and scored for healing 4, 5 and 6 days after treatment with acyclovir ointment or the placebo vehicle.

	Number Healed/Total Wounds		
Treatment Group	**Day 4**	**Day 5**	**Day 6**
Untreated	0/15	ND	15/15
PEG 4x/day x 5 days	0/16	15/28*	14/14
5% ACV 4x/day x 5 days	0/14	7/28*	12/16

*p = 0.03

They concluded that topical acyclovir caused a small decrease in the rate of epidermal wound healing in their pig model.

Eye Irritation
An ointment formulation of acyclovir was tested for eye irritation in rabbits. Concentrations of 1%, 3%, and 6% acyclovir in petrolatum base were instilled into the conjunctival sac 5 times daily at 90-minute intervals for 21 consecutive days. Groups of 8 rabbits each were dosed with each concentration, the vehicle and with balanced saline. Eye irritation was evaluated by gross observation and biomicroscopic, funduscopic and histologic examinations. None of these methods of examination revealed a significant potential for ophthalmic toxicity.

Ocular Penetration
The instillation of a 1 cm ribbon of a 3% acyclovir ophthalmic ointment in the rabbit eye resulted in biologically and statistically significant levels of drug in the aqueous humor of the eye within ½ hour. The maximum levels [mean values of 0.48 and 0.57 mcg/mL (2.15 and 2.53 mcM)] occurred at 1 and 2 hours after treatment with a rather marked decrease by the fourth hour [mean value 0.13 mcg/mL (0.59 mcM)]. Plasma levels of acyclovir, where present at detectable amounts, were below 0.23 mcg/mL (1 mcM). In 11 of 15 rabbits the plasma levels were below 0.06 mcg/mL (0.25 mcM) (limit of detectability for the assay procedure).

The study shows that acyclovir can penetrate the corneal surface and produce therapeutic levels of the drug in the aqueous humor.

Corneal Wound Healing
Masked controlled rabbit studies were done to determine the toxic effects on corneal wound healing of 3% acyclovir and 0.1% idoxuridine (IDU) in therapeutically effective concentrations. Acyclovir was found to have no significant detrimental effect (in comparison to controls) on the quality of regenerating epithelium or the re-epithelialization of epithelial wounds. IDU treatment of eyes resulted in significant toxic changes in the regenerating epithelium (clinically and histologically) with a significant delay in epithelial wound healing in comparison to control or acyclovir-treated eyes. Acyclovir had no significant effect on the collagen content of stromal wounds as measured by hydroxyproline assay. IDU caused a reduction in collagen content not significantly different from controls but significantly lower than acyclovir.

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