PRODUCT MONOGRAPH

Pr Acyclovir Sodium Injection

(50 mg Acyclovir/mL)

House Standard

Antiviral Agent

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CANADA L4L 8K8

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50 mg Acyclovir/mL

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THERAPEUTIC CLASSIFICATION

Antiviral

ACTION AND CLINICAL PHARMACOLOGY

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, herpes virus-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 herpes virus-specified DNA polymerase. Acyclovir is selectively converted to its active form in herpes virus 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 herpes virus replication. No effect on latent nonreplicating 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.

Pharmacokinetics
The pharmacokinetics of acyclovir has been evaluated in 95 patients (9 studies). Results were obtained in adult patients with normal renal function during Phase I/II studies after single doses ranging from 0.5 to 15 mg/kg and after multiple doses ranging from 2.5 to 15 mg/kg every 8 hours. Pharmacokinetics was also determined in pediatric patients with normal renal function ranging in age from 1 to 17 years, at doses of 250 mg/m^2 or 500 mg/m^2 every 8 hours. In these studies, dose-independent pharmacokinetics is observed in the range of 0.5 to 15 mg/kg. Proportionality between dose and plasma levels is seen after single doses or at steady state after multiple dosing.

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

INDICATIONS AND CLINICAL USE

Acyclovir Sodium Injection is indicated for the treatment of initial and recurrent mucosal and cutaneous herpes simplex (HSV-1 and HSV-2) infections and varicella-zoster (shingles) infections in immunocompromised adults and children. It is also indicated for severe initial episodes of herpes simplex infections in patients who may not be immunocompromised. Use in other herpes group infections is the subject of ongoing study.

The indications are based on the results of a number of double-blind, placebo-controlled studies which examined changes in virus excretion, total 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 the therapy is begun at the earliest possible moment.

Herpes Simplex Infections in Immunocompromised Patients
A multicentre trial using intravenous acyclovir at a dose of 250 mg/m^2 every 8 hours infused over 1 hour (750 mg/m^2/day) for 7 days was conducted in 98 immunocompromised patients with oro-facial, esophageal, genital and other localized infections (52 treated with acyclovir and 46 with placebo). Acyclovir significantly decreased virus excretion, reduced pain, and promoted scabbing and rapid healing of lesions.

Initial Episodes of Herpes Genitalis
A controlled trial was conducted in 28 patients with initial severe episodes of herpes genitalis with an acyclovir dosage of 5 mg/kg, infused over 1 hour, every 8 hours for 5 days (12 patients treated with acyclovir and 16 with placebo). Significant treatment effects were seen in elimination of virus from lesions and in reduction of healing times.

In a similar study, 15 patients with initial episodes of genital herpes were treated with acyclovir 5 mg/kg, infused over 1 hour, every 8 hours for 5 days, and 15 with placebo. Acyclovir decreased the duration of viral excretion, new lesion formation, duration of vesicles and promoted more rapid healing of all lesions.

Varicella-Zoster Infections in Immunocompromised Patients
A multicentre trial using intravenous acyclovir at a dose of 500 mg/m^2 every 8 hours for 7 days was conducted in immunocompromised patients with zoster infections (shingles). Ninety-four patients were evaluated (52 patients were treated with acyclovir and 42 with placebo). Acyclovir halted progression of infection as determined by significant reductions in cutaneous dissemination, visceral dissemination, or the proportion of patients deemed treatment failures.

A comparative trial of acyclovir and vidarabine was conducted in 22 severely immunocompromised patients with zoster infections. Acyclovir was shown to be superior to vidarabine as demonstrated by significant differences in the time of new lesion formation, the time to pain reduction, the time to lesion crusting, the time to complete healing, the incidence of fever and the duration of positive viral cultures. In addition, cutaneous dissemination occurred in none of the 10 acyclovir recipients compared to 5 of the 10 vidarabine recipients who presented with localized dermatomal disease.

Healing Process
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 excretion.

Diagnosis
Whereas cutaneous lesions associated with herpes simplex and varicella-zoster infections are often pathognomonic, Tzanck smears prepared from lesion exudate or scrapings may assist in diagnosis. Positive cultures for herpes simplex virus offer the only absolute means for confirmation of the diagnosis. Appropriate examinations should be performed to rule out other sexually transmitted diseases. The Tzanck smear does not distinguish varicella-zoster from herpes simplex infections.

CONTRAINDICATIONS

Acyclovir Sodium Injection is contraindicated in patients who have hypersensitivity to the drug.

WARNINGS

Acyclovir Sodium Injection is for slow intravenous infusion only. Intravenous infusions must be given over a period of at least 1 hour to reduce the risk of renal tubular damage (see **PRECAUTIONS** and **DOSAGE AND ADMINISTRATION**).

In severely immunocompromised patients, the physician should be aware that prolonged or repeated courses of acyclovir may result in selection of resistant viruses associated with infections which may not respond to continued acyclovir therapy. This, however, remains to be clearly established and should be considered as a factor when undertaking therapy. The effect of the use of acyclovir on the natural history of herpes simplex or varicella-zoster infection is unknown.

PRECAUTIONS

Precipitation of acyclovir crystals in renal tubules can occur if maximum solubility (2.5 mg/mL at 37°C in water) is exceeded. This phenomenon is reflected by a rise in serum creatinine and blood urea nitrogen (BUN) and a decrease in creatinine clearance. With sufficient renal tubular compromise, urine output decreases.

Acute increases in serum creatinine and decreased creatinine clearance have been observed in

humans receiving intravenous acyclovir who were poorly hydrated; or receiving concomitant nephrotoxic drugs (e.g., amphotericin B and aminoglycoside antibiotics); or had pre-existing renal compromise or damage; or had the dose administered by rapid intravenous injection (less than 10 minutes). Observed alterations in renal function have been transient, in some instances resolving spontaneously without change in acyclovir dosing regimen. In other instances, renal function improved following increased hydration, dosage adjustment, or discontinuation of acyclovir therapy.

Administration of acyclovir by intravenous infusion must be accompanied by adequate hydration. Since maximum urine concentration occurs within the first 2 hours following infusion, particular attention should be given to establishing sufficient urine flow during that period in order to prevent precipitation in renal tubules. Recommended urine output is ≥ 500 mL/g of drug infused.

When dosage adjustments are required, they should be based on estimated creatinine clearance (see **DOSAGE AND ADMINISTRATION**).

Approximately 1% of patients receiving intravenous acyclovir have manifested encephalopathic changes characterized by either lethargy, obtundation, tremors, confusion, hallucinations, agitation, seizures or coma. Acyclovir should be used with caution in those patients who have underlying neurologic abnormalities and those with serious renal, hepatic, or electrolyte abnormalities or significant hypoxia. It should also be used with caution in patients who have manifested prior neurologic reactions to cytotoxic drugs or those receiving concomitant intrathecal methotrexate or interferon.

Nursing Mothers
Acyclovir is excreted in human milk. Caution should therefore 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, acyclovir 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.

No data exist at this time, that demonstrate that the use of acyclovir will prevent transmission of herpes simplex infection to other persons.

Consideration should be given to an alternative treatment regimen if after 5 days of treatment there is no expected clinical improvement in the signs and symptoms of the infection.

Strains of herpes simplex virus which are less susceptible to acyclovir have been isolated from herpes lesions and have also emerged during intravenous treatment with acyclovir.

Drug Interactions
Co-administration of probenecid with intravenous acyclovir has been shown to increase the mean

half-life and the area under the concentration-time curve. Urinary excretion and renal clearance were correspondingly reduced.

ADVERSE REACTIONS

The adverse reactions listed below have been observed in controlled and uncontrolled clinical trials in approximately 700 patients who received acyclovir at approximately 5 mg/kg (250 mg/m^2) and approximately 200 patients who received approximately 10 mg/kg (500 mg/m^2).

The most frequent adverse reactions reported during intravenous acyclovir administration were inflammation or phlebitis at the injection site in approximately 9% of the patients, and transient elevations of serum creatinine or BUN in 5% to 10% [the higher incidence occurred usually following rapid (< 10 minutes) intravenous infusion]. Nausea and/or vomiting occurred in approximately 7% of the patients (the majority occurring in nonhospitalized patients who received 10 mg/kg). Itching, rash or hives occurred in approximately 2% of patients. Elevation of transaminases occurred in 1 to 2% of patients.

Approximately 1% of patients receiving intravenous acyclovir have manifested encephalopathic changes characterized by either lethargy, obtundation, tremors, confusion, hallucinations, agitation, seizures or coma (see **PRECAUTIONS**).

Adverse reactions which occurred at a frequency of less than 1% and which were probably or possibly related to intravenous acyclovir administration were: anemia, anuria, hematuria, hypotension, edema, anorexia, lightheadedness, thirst, headache, diaphoresis, fever, neutropenia, thrombocytopenia, abnormal urinalysis (characterized by an increase in formed elements in urine sediment) and pain on urination.

Other reactions have been reported with a frequency of less than 1% in patients receiving acyclovir, but a causal relationship between acyclovir and the reaction could not be determined. These include pulmonary edema with cardiac tamponade, abdominal pain, chest pain, thrombocytosis, leukocytosis, neutrophilia, ischemia of digits, hypokalemia, purpura fulminans, pressure on urination, hemoglobinemia and rigors.

SYMPTOMS AND TREATMENT OF OVERDOSAGE

Overdose has been reported following administration of bolus injections, or inappropriately high doses, and in patients whose fluid and electrolyte balance was not properly monitored. This has resulted in elevations in BUN, serum creatinine and subsequent renal failure. Lethargy, convulsions and coma have been reported rarely. Precipitation of acyclovir in renal tubules may occur when the solubility (2.5 mg/mL) in the intratubular fluid is exceeded (see **PRECAUTIONS**).

A 6-hour hemodialysis results in a 60% decrease in plasma acyclovir concentration. Data

concerning peritoneal dialysis are incomplete but indicate that this method may be significantly less efficient in removing acyclovir from the blood. In the event of acute renal failure and anuria, the patient may benefit from hemodialysis until renal function is restored (see **DOSAGE AND ADMINISTRATION**).

DOSAGE AND ADMINISTRATION

Caution: Acyclovir Sodium Injection is for slow intravenous infusion only, over a period of at least 1 hour.

Herpes Simplex Infections
Mucosal and Cutaneous Herpes Simplex (HSV-1 and HSV-2) in Immunocompromised Patients:

Adults: 5 mg/kg infused at a constant rate over at least 1 hour, every 8 hours for 7 days in adult patients with normal renal function.

Children: In children under 12 years of age, equivalent plasma concentrations are attained by infusing 250 mg/m^2 at a constant rate over at least 1 hour, every 8 hours for 7 days.

Severe Initial Clinical Episodes of Herpes Genitalis in Immunocompetent Patients:
The same dose given above, administered for 5 days.

Varicella Zoster Infections
Zoster in Immunocompromised Patients:

Adults: 10 mg/kg infused at a constant rate over at least 1 hour, every 8 hours for 7 days in adult patients with normal renal function.

Children: In children under 12 years of age, equivalent plasma concentrations are attained by infusing 500 mg/m^2 at a constant rate over at least 1 hour, every 8 hours for 7 days.

Obese patients should be dosed at 10 mg/kg (Ideal Body Weight).

A maximum dose equivalent to 500 mg/m^2 every 8 hours should not be exceeded for any patient.

Patients with Acute or Chronic Renal Impairment
Use the recommended doses and method of administration; and adjust the dosing interval as indicated in the following table.

Creatinine Clearance (mL/min/1.73 m^2)	Percent of Recommended Dose	Dosing Interval (hours)

> 50	100	8
25 to 50	100	12
10 to 25*	100	24
0 to 10*	50	24 to 48

*Hemodialysis: For patients who require hemodialysis, the mean plasma half-life of acyclovir during dialysis is approximately 5 hours, which results in a 60% decrease in plasma concentrations following a 6-hour dialysis period. Recommended doses should be administered every 24 to 48 hours, and after hemodialysis.
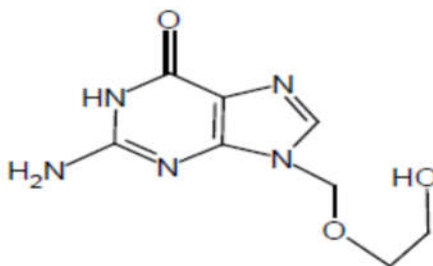
PHARMACEUTICAL INFORMATION

Drug Substance

Proper[*] Name: Acyclovir

Chemical Name: 2-amino-9-[(2-hydroxyethoxy)methyl]-1,9-dihydro-6*H*-purin-6-one

Chemical Structure:

Molecular Formula: $C_8H_{11}N_5O_3$

Molecular Weight: 225.2 g/mol

Description: A white or almost white, crystalline powder.
Slightly soluble in water, very slightly soluble in alcohol (96%), practically insoluble in heptane. It dissolves in dilute solutions of mineral acids and alkali hydroxides.

pH and pKa values: pH: 5.93 and pKa: 2.27 and 9.25

Composition of the Drug product: *Acyclovir sodium is prepared in situ with the aid of sodium hydroxide.

Each mL of drug product solution contains 50 mg / mL acyclovir (as the sodium salt) in water for injection. pH of the solution is between 10.85 and 11.50.

STABILITY AND STORAGE RECOMMENDATIONS

Store between 15 and 25°C.

Diluted Solutions for Intravenous Infusion
The calculated dose of the solution should be removed and added to an appropriate intravenous solution listed below at a volume selected for administration during each 1-hour infusion.
Infusion concentrations exceeding 10 mg/mL are not recommended.

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[*] Acyclovir sodium is obtained by an in-situ reaction.

Since the solution does not contain any preservatives, any unused portion of the vial should be discarded.

Solutions for Intravenous Infusion:
5% Dextrose Injection
5% Dextrose and 0.9% Sodium Chloride Injection
Normal Saline Injection
Lactated Ringer's Injection

Once diluted, the admixtures are to be administered within 24 hours of the initial preparation. The admixtures are not to be refrigerated.

The diluted solution should be inspected visually for discolouration, haziness, particulate matter and leakage prior to administration. Discard unused portion.

Incompatibility: Acyclovir Sodium Injection should not be added to biologic or colloidal fluids (e.g. blood products, protein hydrolysates or amino acids, fat emulsions).

AVAILABILITY OF DOSAGE FORMS

Dosage form	Solution
Strength	**50 mg/mL**
Label Claim	Each ml contains Acyclovir Sodium equivalent to 50 mg Acyclovir
Description	Clear, colourless solution filled in clear glass vials.
Composition	**Non-medicinal Ingredients:** Sodium Hydroxide and Water for injection
Packaging	Available as 500 mg/10 mL and 1000 mg/20 mL SINGLE USE Type I glass vials in boxes/carton of 10 vials.

VIROLOGY

Spectrum of Activity *In Vitro*
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 0.02 µg/mL (plaque reduction in Vero cells) to 5.9 - 13.5 µg/mL (plaque reduction in green monkey kidney [GMK] cells). The ID_{50} against HSV-2 ranges from 0.01 µg/mL to 9.9 µg/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 ≤ 0.9 µg/mL acyclovir and 50% of all isolates were sensitive to ≤ 0.2 µg/mL acyclovir. For HSV-2 isolates, 90% were sensitive to ≤ 2.2 µg/mL and 50% of all isolates were sensitive to ≤ 0.7 µg/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 (0.1 µg/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 - 1.53 µg/mL (yield reduction, human foreskin fibroblasts) to 1.85 - 3.98 µg/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 1.5 µg/mL acyclovir. CMV is relatively resistant to acyclovir with ID_{50} values ranging from 2.3 - 17.6 µg/mL (plaque reduction, HEF cells) to 1.82 – 56.8 µg/mL (DNA hybridization, HEF cells). The latent state of the human herpesviruses is not known to be sensitive to acyclovir.

Resistance
Prolonged exposure of herpes simplex virus (HSV) to subinhibitory concentrations (0.1 µg/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 herpesvirus, 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.

PHARMACOLOGY

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

Similar concentrations are achieved in pediatric patients over 1 year of age when doses of 250 mg/m^2 BSA are given intravenously 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 of intravenously administered ^{14}C-labelled drug in man. The only significant urinary metabolite is 9-carboxymethoxy-methylguanine.

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 intravenous acyclovir is dependent on renal function as shown below.

Creatinine Clearance (mL/min/1.73 m² BSA*)	Half-Life (hr)	Total Body Clearance (mL/min/1.73 m² BSA)
> 80	2.4	332
50 - 80	2.9	251
15 - 50	3.7	185
0 (Anuric)	18	26

*Body Surface Area

The half-life and total body clearance of intravenous acyclovir in pediatric patients over 1 year of age is similar to adults with normal renal function. Additional data are needed to fully define the pharmacokinetics of intravenous acyclovir in premature infants.

Plasma Concentration of Acyclovir in Neonates

Dose	5 mg/kg q8h	10 mg/kg q8h	15 mg/kg q8h
Mean Peak conc.	30 µM ± 9.9 equivalent to 6.75 µg/mL	61.2 µM ± 18.3 equivalent to 13.8 µg/mL	86.1 µM ± 23.5 equivalent to 19.4 µg/mL
Mean trough conc.	5.3 µM ± 3.4 equivalent to 1.19 µg/mL	10.1 µM ± 8.4 equivalent to 2.27 µg/mL	13.8 µM ± 11.1 equivalent to 3.1 µg/mL

Principal Pharmacokinetic Parameters in Neonates

Cl_{tot} (mL/min/1.73 m²)	-	105 ± 42
$t_{1/2}$ (h)	-	4.05 ± 1.22
Vd_{ss} (L/1.73 m²)	-	28.8 ± 9.3

Pharmacokinetic Parameters in Patients with End-Stage Renal Disease

Terminal $t_{1/2}$ (h)	-	19.5 ± 5.9
V_1 (L/1.73 m²)	-	15.3 ± 8.1
Vd_{ss} (L/1.73 m²)	-	41.2 ± 2.3
V_1/Vd_{ss} x 100 (%)	-	37 ± 19.9
Cl_{tot} (mL/min/1.73 m²)	-	28.6 ± 9.5
Kel (L/h)	-	0.15 ± 0.09
Terminal dialysis $t_{1/2}$ (h)	-	5.73 ± .85
Coefficient of dialysis extraction	-	0.45 ± 0.12

Acyclovir Dosing in Patients with End-Stage Renal Disease

	Changing Dosage	Changing Interval
Loading Dose	37% of the standard dosage* ($93 - 185$ mg/m^2)	Full standard dose ($250 - 500$ mg/m^2)
Maintenance Dose	14% of the standard dosage every 8 hours ($35 - 70$ mg/m^2)	Full standard dose every 48 hours ($250 - 500$ mg/m^2)
Post-Dialysis	60 - 100% loading dose	60 – 100% standard dose
Potential Advantages	Minimizes fluctuations between peaks and troughs	Less frequent administration

*   Standard acyclovir dosage (patients with normal renal function)
   = $250 - 500$ mg/m^2 initially and every eight hours.

TOXICOLOGY

Acute Toxicity Studies
Adult Mice and Rats: The acute toxicity of acyclovir was determined as follows:

Species	Sex	Route	LD$_{50}$ (mg/kg)	95% Conf. Level	Signs
Mouse	M	Oral	> 10,000	-	None
Rat	M	Oral	> 20,000	-	None
Mouse	M	IV	405	-	Ataxia Depression
Rat	M	IV	> 600	-	None
Mouse	M	IP	1,454	1,323 - 1,662	Sedation
Mouse	F	IP	999	670 - 1,364	Sedation
Rat	M	IP	1,305	512 - 1,733	Sedation
Rat	F	IP	1,210	504 - 1,580	Sedation

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 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.

| | LD_{50} (mg/kg body weight) | |
Age When Treated	Males	Females
3 Days	1,070	1,281
10 Days	790	496
28 Days	678	750
71 Days	650	1,477

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.

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) µg/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 µg/mL (200 to 1127 µM)] 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 µg/mL (100 to 200 µM) and was only marginally toxic and nearly a "no effect" dose.

Acyclovir Concentrations	
Dose mg/kg b.i.d., s.c.	Plasma (µg/mL)
25 N = 8	22.5 - 45
50 N = 8	45 - 254
100 N = 8	45 - 254

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).

In a second intravenous study, two groups of 8 purebred Beagle dogs (4 males and 4 females) were given acyclovir by bolus injection at dose levels of either 10 or 20 mg/kg, b.i.d. for 31 or 32 consecutive days. A similar group of dogs, serving as controls, were dosed with a 0.9% NaCl solution.

Signs of toxicity were limited to increased water intake and urine output volumes that occurred at

the end of the dosing period in dogs given 20 mg/kg, b.i.d. The increased urine output volumes were accompanied by reduction in urine specific gravity and osmolality. No unusual changes were observed in the general health of the animals.

Plasma acyclovir concentrations were similar on the first and 29th dose days. Averaged plasma acyclovir concentrations 0.5 hours after either of the daily doses were 37 and 79 µM for the low and high dose groups respectively.

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 - 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).

Chronic Toxicity Studies
Lifetime Oral Toxicity Study in Rats Given Acyclovir by Gastric Intubation: Charles River CD (Sprague-Dawley) rats were given suspensions of acyclovir by gavage. 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. The remaining rats were

dosed each day until natural mortality decreased a group size to approximately 20% of the number of animals of that sex present in the test groups when the study was started. All remaining rats were killed and necropsied when the 20% cut-off point was reached. This was during week 110 for the male rats and week 122 for the female rats. Tissues from control rats and those in the high-dose group were evaluated by light microscopy. Tissues from rats in the low and mid-dose groups having masses, nodules or unusual lesions were also examined by light microscopy. 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. Plasma samples were collected 1.5 hours after dosing on days 7, 90, 209, 369, 771 (males only) and 852 (females only). Mean plasma levels found in high dose males (450 mg/kg/day) at the times indicated above were as follows: 1.54, 1.63, 1.39, 1.60 and 1.70 µg/mL (6.84, 7.26, 6.17, 7.10 and 7.56 µM). Corresponding mean plasma levels for the high-dose females for the corresponding time periods were 1.76, 2.38, 2.12, 1.71 and 1.81 µg/mL (7.82, 10.58, 9.44, 7.62 and 8.03 µM). 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 and there was no evidence that acyclovir affected survival. 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.

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 µg/mL (6.84, 7.26, 6.17, 7.10 and 7.56 µM) 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 µg/mL (7.82, 10.58, 9.44, 7.62 and 8.03 µM).

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 µg/mL (12.59, 14.10 and 8.10 µM) at days 90, 365 and 541, respectively. Corresponding mean values for the high-dose females were 9.81, 5.85 and 4.0 µg/mL (43.60, 26.0 and 17.79 µM).

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.

Chronic 12-Month Oral 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 µg/mL (106.6 µM) for high-dose males and 17.4 µg/mL (77.2 µM) 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 sequela. 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 for one year. The "no dose effect" dose level of acyclovir was 15 mg/kg/day (5 mg/kg t.i.d.); however, the only adverse effects at 30 or 60 mg/kg/day were changes in nails and footpads (30 and 60 mg/kg/day) and mild gastrointestinal signs (60 mg/kg/day).

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 (mc/mL)	Amniotic Fluid (µg/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.

In a second study, female Wistar rats (35/group) were given acyclovir at 0, 6.25, 12.5, or 25 mg/kg twice daily subcutaneously from days 7 to 17 of pregnancy, with two-thirds of dams terminated on day 20 of pregnancy and the remainder allowed to deliver and rear their young. There was no maternal toxicity during the treatment period or subsequently that could be attributed to treatment, although increased water consumption was observed in the high dosage group. The latter observation was not correlated with any adverse effects in the dams or their offspring.

No aspects of pregnancy or of embryonic or fetal development were meaningfully different from those in controls in the pregnancies terminated on day 20.

Pregnancies allowed to continue to term were of significantly longer duration in females treated with the highest dose than in controls. This, however, may have been artifactual because 1) the control value was less than the historical value for the laboratory, and 2) there was no indication that these or other similarly treated pregnancies were adversely affected by this dosage of acyclovir.

Newborn pups from treated dams did not differ from controls and subsequent development was within normal ranges, although some performances in the E-maze were marginally statistically elevated but not in a dose-related pattern. Dilated renal pelves and occasional structural defects occurred throughout the groups but did not display dose-dependent incidence. There may have been a tendency for dilatation of renal pelves to be more prevalent in groups in which postnatal growth was to some extent slowed, but this was neither consistent, dose-related, nor statistically significant.

In summary, there was no acceptable evidence that treatment of pregnant rats throughout the period of organogenesis with the dosage levels of acyclovir used here caused deviations from the normal range of embryonic, fetal or postnatal development. Related aspects of pregnancy, parturition and lactation also were within normal ranges.

In a third study, groups of 25 pregnant Sprague-Dawley rats were given subcutaneous doses of acyclovir at 6.25, 12.5 or 25 mg/kg b.i.d. on the 6th through the 15th days of gestation. Their fetuses were taken by cesarean section on the 20th day of gestation and examined for gross, visceral and skeletal abnormalities. There were no signs of teratogenesis or other fetal toxicity. Clinical signs in the dams consisted of marginally decreased body weights at all dose levels and cutaneous scabs and alopecia (dose-related incidence, size and duration of the scabs). The mean concentration of acyclovir in plasma taken from high dose dams samples 15 minutes after the second dose given on the 6th days of gestation was 80 µM (18 µg/mL).

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 (µg/mL)	Amniotic Fluid (µg/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 = 4	$0.39 \pm 0.12^*$	6.16 ± 4.25	0.315 (1.40 ± 0.19)
*N = 5			

Reproduction - Fertility: Acyclovir does not impair fertility or reproduction in mice (450 mg/kg/day, p.o.) or in rats (25 mg/kg/day, s.c.). In female rabbits treated subcutaneously with acyclovir subsequent to mating, there was a statistically significant decrease in implantation efficiency but no concomitant decrease in litter size at a dose of 50 mg/kg/day. No effect upon implantation efficiency was observed when the same dose was administered intravenously. The intravenous administration of 100 mg/kg/day, a dose known to cause obstructive nephropathy in rabbits, caused a significant increase in fetal resorptions and a corresponding decrease in litter size. However, at a maximum tolerated intravenous dose of 50 mg/kg/day in rabbits, there were no drug-related reproductive effects. Intraperitoneal doses of 320 or 80 mg/kg/day acyclovir given to rats for 1 and 6 months, respectively, caused testicular atrophy. Testicular atrophy was persistent through the 4-week postdose recovery phase after 320 mg/kg/day; some evidence of recovery of sperm production was evident 30 days postdose.

Developmental Toxicity Studies
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 µg/mL (440.5 µM) 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.

Mutagenicity and other Short-Term Studies
Acyclovir has been tested for mutagenic potential in a number of *in vitro* and *in vivo* systems:

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$^{+/-}\longrightarrow$ TK $^{-/-}$); additional studies were performed at the HGPRT locus and at 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 - 2,400 μg/mL) concentrations.

(By comparison, acyclovir peak plasma levels following intravenous infusion at recommended dose levels are 20 μg/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 µg/mL (222 µM) 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 µg/mL and 64 µg/mL for 18 hours exposure did not induce any morphologically-transformed foci among C3H/10T 1/2 cells treated *in vitro* in the absence of exogenous metabolic activation.

Acyclovir, at concentrations of 62.5 and 125 µg/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 µg/mL for 48 hours exposure - acyclovir caused a significant increase in the incidence of chromosome breakage. There was also a significant dose-related decrease in mitotic index with exposure to acyclovir.

Acyclovir, at doses of 25 and 50 mg/kg/day i.p. for 5 consecutive days, did not produce a dominant lethal effect in male BKA (CFLP) mice. Further, there was no evidence of a dominant lethal effect on Charles River CD-1 (ICR) male and female mice treated orally at dose levels of 50, 150 and 450 mg/kg/day.

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 µg/mL (2.72 mM) which is 30 times higher than the mean peak adult human plasma levels at 10 mg/kg q8h i.v. and 45 times the mean peak neonatal plasma level at a similar dose.)

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.

Thus, all these studies showed that acyclovir does not cause single-gene mutations but is capable of breaking chromosomes.

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 µg/mL (600 µM). The compound inhibited rosette formation approximately 50% at 0.9 µg/mL (4 µM).

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, phytohemagglutinin (PHA) and concanavalin (Con A), and only at concentrations of drug above 50 µg/mL (222 µM) 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 µg/mL (890 µM) where there has already been demonstrated to be a direct cytotoxic effect. 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 - 22.5 µg/mL (50 - 100 µM) 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 phytohemagglutin or three different herpesvirus antigens. A linear dose-response curve was observed with these cells, and their proliferation was 50% inhibited by 22.5 µg/mL (100 µM) acyclovir. Inhibition was exerted on T-cell proliferation without apparent effect on the release of lymphokines or on monocyte function.

It should also be mentioned that there was no evidence of adverse effects on the immune system in the detailed subchronic and chronic animal tests covered earlier in this summary except at excessively high doses (50 to 100 mg/kg b.i.d.) in dogs where marked lymphoid hypoplasia occurred.

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