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**The effect of Famciclovir (FCV) on herpes simplex virus type 1 (HSV-1) corneal disease, latency and reactivation in the rabbit eye model.** JM Loutsch[1], B Sainz, Jr. [1], ME Marquart[1], X Zheng[1], HK van der Keyl[2], JM Hill[1] and R Tal-Singer[2]. [1]LSU Eye Center, New Orleans, LA, [2]SmithKline Beecham Pharmaceuticals, Collegeville, PA.

FCV, the oral pro-drug of penciclovir, is efficacious in the treatment of acute herpes zoster and recurrent genital herpes as well as systemic infections of HSV-1. Although FCV has been studied in ophthalmic zoster, FCV has not been used to treat ocular HSV infections. Our objective was to evaluate the efficacy of b.i.d. FCV as a treatment for HSV-1 epithelial keratitis. New Zealand white rabbits were inoculated with HSV-1 strain 17syn+. On post-inoculation (PI) day 3, rabbits were randomly assigned into four experimental groups: placebo (water), 120 mg/kg, 250 mg/kg and 500 mg/kg FCV. Study endpoints included survival, severity of keratitis evaluated using slit-lamp biomicroscopy (SLE), establishment of latency, spontaneous and epinephrine-induced reactivation. Treatment with FCV resulted in significant and dose-dependent improvement in keratitis SLE scores during acute infection as well as a trend for prolonged survival. Regardless of treatment, all groups exhibited the high rates of spontaneous as well as epinephrine induced reactivation characteristic of 17syn+ (75-100% rabbits positive). Real time quantitative PCR analysis of rabbit corneas and trigeminal ganglia demonstrated no drug-induced difference in the amount of HSV DNA or RNA. In a repeat experiment, the efficacy of 250 mg/kg of FCV was compared to topical treatment with 1% trifluoridine (TFT, 5 times per day for 8 days). Although TFT treatment was more effective at reducing eye disease, FCV treated rabbits tended to have a better survival rate. When FCV treatment was started 24 h after inoculation the survival rate was significantly higher ($P = 0.0253$). In summary, oral treatment significantly reduces the severity of established corneal lesions, improves survival and therefore may be beneficial in reducing the morbidity of HSV keratitis in patients.

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Orofacial Herpes Simplex Virus Type 1 (HSV-1) Infection in Mice: A Model for Herpes Labialis. J. Palmer, R.J. Rybak, and E.R. Kern. The University of Alabama School of Medicine, Birmingham, Ala., USA.

It has been estimated that there are over 40 million people in the U.S. with herpes labialis and therapy for this disease is less than optimal. We have utilized an orofacial HSV-1 infection in mice as a model for evaluation of new therapies for this disease. Immunocompetent hairless SKH-1 mice were anesthetized, the snout lightly abraded, and a dacron applicator soaked in 2 x $10^6$ pfu/ml of HSV-1 was applied to the tissue. The time of appearance and severity of lesions was recorded daily and the area was sampled for quantitation of virus replication. Lesions first appeared on day 5, reached maximum severity on day 9 and healed completely in survivors by day 18. Using this inoculum there was 70% mortality with a mean day of death of 9.2 days. Infectious HSV-1 was first detected on day 2, reached peak titer of $10^5$ pfu/g sample on day 5 and cleared by days 7-10. To determine the extent of the HSV-1 infection, groups of mice were sacrificed on days 1-7, 10, and 21 and visceral and CNS tissues were removed, homogenized, and assayed for HSV-1. In non-CNS tissue, virus was isolated only from salivary gland and a small amount in lung on day 5. In brain tissue, infectious virus was detectable in the trigeminal ganglia within 24h, the olfactory lobes by day 2, the pons/medulla by day 3, the diencephalon and cerebellum by day 4, and the cerebrum on days 5 and 6. To validate this infection as a suitable model for evaluation of new antiviral therapies, mice were infected as described above and topical treatment with 5% Acyclovir (ACV), 5% ACV-monophosphate (ACVMP), or 1% Penciclovir (Denavir, PCV) was initiated 24, 48, or 72h after infection and continued 3 times daily for 7 days. Treatment with ACV or ACVMP significantly reduced viral replication and lesion formation when applied as late as 72h post infection. In contrast, PCV was not effective even when treatment was begun 24h after infection. These results indicate that the orofacial HSV-1 infection of mice shares many clinical and virological features with herpes labialis, that the infection can be ameliorated with an effective drug such as ACV, and should be a good model for evaluating new therapies for oral and cutaneous HSV infections.

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**In vitro and in vivo Activity of N,N′-Bisheteryl Derivatives of Dispirotripiperazine**

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Previously, we could show that N,N′-bisheteryl derivatives of dispirotripiperazine (DSTP) are well tolerated in vitro and very effectively inhibit the replication of aciclovir- and foscarnet-sensitive as well as resistant herpes simplex virus type 1 (HSV1) strains. As shown for sulfated polyanions, the mechanism of antiviral activity is based on the inhibition of virus adsorption to the cell surface. In contrast to sulfated polyanions e. g. pentosan polysulfate (PPS), the pre-treatment of cells but not the pre-incubation of the virus with DSTP is sufficient for the prevention of virus infection. Prolonged pre-incubation of cell monolayers with the compounds before virus inoculation significantly decreases the 50 % inhibitory concentration. When the treatment preceded viral challenge by 1 to 4 hrs, protection was also seen but higher concentrations are needed to prevent a partial loss of activity. In combination with PPS a strong antagonism was observed corroborating the adverse mode of action of both compounds. Like PPS, DSTP prevented other virus infections in which heparansulfates serve as receptor e. g. HIV, HSV2, cytomegalovirus, and respiratory syncytial virus suggesting that heparansulfates may be involved in the antiviral mechanism.

The most active in vitro compounds were examined for acute toxicity and prevention of acute herpes infections in mice. The treatment with DSTP two or one hrs prior to, immediately prior to, and two hrs after HSV1 inoculation dose-dependent increased the mean survival time and decreased the number of mice that died. Further animal experiments seems warranted to evaluate the potential of these compounds in chronic virus infections and as topical microbicides to prevent the spread of sexually transmitted virus diseases.

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**Effects of Guanosine Nucleoside Analogues on Cell Death During HSV Infection *in vivo* and *in vitro*.**

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We previously showed that ganciclovir (GCV) and penciclovir (PCV) but not acyclovir (ACV) induced apoptosis of HSVTK-transformed BHK cells. We now consider their effects on HSV-1 infected cells *in vitro* and *in vivo*. BHK cells were infected at MOI 0.5 and treated with ACV, PCV or GCV. At various times p.i. cells were double-stained for flow cytometry analysis using anti-HSV-FITC antibody or propidium iodide. Viable and non-viable $HSV^{+ve}$ and $HSV^{-ve}$ cell populations were compared. The drugs reduced the number of HSV antigen-expressing cells in all cases. However, treatment also produced more $antigen^{-ve}$, non-viable cells, suggesting either a "bystander effect" or reduced production of viral proteins to below background. These results were extended *in vivo*. Balb/c mice, infected in the ear pinna with HSV-1 were treated with 25mg/kg bid ACV, PCV or GCV i.p. or with famciclovir (FCV) or valaciclovir (VACV) at 5mg/ml in the drinking water. On day 6 p.i. serial cryosections of brain stem were stained for virus antigen or free 3'OH DNA strand termini (TUNEL assay). More cells were $TUNEL^{+ve}$ than $antigen^{+ve}$ with or without therapy. The areas of $antigen^{+ve}$ and $TUNEL^{+ve}$ foci in the brain stem were expressed as a ratio and, in all cases, drug treatment increased the proportion of $TUNEL^{+ve}$ foci compared to untreated controls, with the highest proportion of $TUNEL^{+ve}$ foci following treatment with FCV. These results have implications for the mechanism of drug action by induction of cell death, as opposed to direct interference with virus replication.