## 99

**Metabolic fate and mode of antiviral action of sorivudine in HSV-1 infected cells.**

**N. Ashida[1], S. Sakata[2], H. Machida[1]. Biology Lab.[1] and Chemistry Lab.[2], Yamasa Corp., Choshi, Japan**

To study the mode of action of sorivudine in addition to the previous findings, we examined uptake of [$^3$H]sorivudine by HSV-1-infected cells and whether [$^3$H]sorivudine taken-up by the cells is incorporated into DNA strand or not. [$^3$H]Sorivudine was selectively taken-up by the infected cells, and sorivudine uptake increased with incubation time by 8 h after infection. Incorporation of [$^3$H]sorivudine, like that of [$^3$H]acyclovir used as control, into the acid-insoluble fraction increased with incubation time from 4 h to 8 h, and was about 10% of total uptake at each time point. When [$^3$H]sorivudine was exposed to the cells immediately after infection, incorporation of [$^3$H]sorivudine into the acid-insoluble fraction increased in portion to duration of [$^3$H]sorivudine-exposure up to 12 h. By denaturation and digestion with nuclease $P_1$, 99% of radioactivity of the DNA, extracted from HSV-1-infected and [$^3$H]sorivudine-treated cells, was converted to low-molecular-weight DNA. HSV-1-infected cells were pulse-labeled with [$^3$H]sorivudine, and DNA obtained from the cells were analyzed by alkaline sucrose gradient centrifugation. The sedimentation profile of labeled DNA was not changed after chasing in isotope-free medium, indicating that the DNA was not chased into high-molecular-weight DNA. They reveal that [$^3$H]sorivudine is actively incorporated at the same period of DNA synthesis in virus replication process, and that [$^3$H]sorivudine taken-up by the infected cells is incorporated into the viral DNA strand and inhibits elongation of the DNA strand.

## 100

**Antiviral efficacies of famciclovir, valaciclovir, and brivudin in disseminated herpes simplex virus type 1 infection in mice**

P. Wutzler, A. Ulbricht, and I. Färber
Institute for Antiviral Chemotherapy, Friedrich-Schiller-University of Jena, Germany

The animal model of necrotic hepatitis caused by HSV-1 infection in juvenile mice was used to compare the efficacy of the oral antiherpes agents famciclovir (FCV), valaciclovir (VACV) and brivudin (BVDU). It allows the measurement of viral replication in the liver by macroscopic lesions and the evaluation of mortality from encephalitis. Mice intravenously inoculated with a highly virulent clinical HSV-1 isolate were orally treated by gavage over a period of 3 days starting on day 2 post infection. The reference drug acyclovir (ACV) was administered subcutaneously. Necrotic hepatitis was significantly ($p<0.01$) reduced by treatment with FCV, VACV and ACV, respectively, at a dose of 50 mg/kg per day divided into 3 doses. No significant effect was achieved with BVDU at 200 mg/kg per day. Treatment with FCV at 50 mg/kg per day, ACV at 100 mg/kg per day, and VACV at 200 mg/kg per day significantly ($p<0.001$) decreased mortality in mice. BVDU treatment at 200 mg/kg per day did not reduce mortality but prolonged significantly ($p<0.05$) the survival time.

## 101

Clinical Evaluation of Carbocyclic Oxetanocin G Eye Drops in the Treatment of Ulcerative Herpetic Keratitis. H. Shiota, K. Nitta, T. Naito, Y. Mimura (Department of Ophthalmology, University of Tokushima, Tokushima City, Japan) & T. Maruyama (Tokushima Bunri University, Tokushima City, Japan)

Herpetic keratitis is one of the most difficult ocular infections to treat. Acyclovir(ACV) ophthalmic ointment is the drug of choice for the treatment. However, the ointment gives an unpleasant feeling and some cases are resistant to ACV. A new compound, carbocyclic oxetanocin G (C. OXT-G), has potent anti-HSV activity and good water-solubility. The clinical evaluation of C.OXT-G eye drops in the treatment of ulcerative herpetic keratitis was done. Forty eyes of 30 patients with typical dendritic or geographic corneal ulcers were treated with 0.1% C.OXT-G eye drops, applied 5 times a day. The eyes were examined at least twice a week until the ulcers healed. The ulcers in 39 eyes healed within 2 weeks. The average healing time of ulcers was 4.9±2.2 days. A geographic ulcer of one eye did not respond to C.OXT-G and was verified due to HSV-2 infection. No adverse reactions were seen during the observation period in this trial. Therefore, C.OXT-G eye drops are excellent and safe in the treatment of herpetic keratitis in humans.

## 102

Efficacy of Glutathione on Experimental HSV-1 Keratitis A.T. Palamara,*C. Nucci, C. Buè,*G.L. Scuderi,*S. Palma, C. D'Agostini and E. Garaci. Depts. of Exp. Medicine and *Surgery, University of Rome Tor Vergata, Rome, Italy.

We previously reported that addition of exogenous Glutathione (GSH) to HSV-1 infected cells was able to inhibit >99% the replication of HSV-1 *in vitro*. Electron microscopic examination and immunoblot analysis of viral proteins confirmed these data. In the present study we evaluated the effect of topical GSH application on experimental acute Herpes keratitis in the rabbit ocular model. 24 rabbits were infected in the right eye by placing a suspension of HSV-1 ($2X10^5$ pfu) into the conjunctival cul-de-sac. Left eyes were used to evaluate ocular toxicity. 12 rabbits received 7% ointment of GSH in methyl hydroxy-propyl cellulose and 12 received only the drug diluent. Fluorescein stained corneas were examined every two days using slit lamp biomicroscope and the number of lesions and the extent of epithelial keratitis was scored. Conjunctival involvement and palpebral edema were also scored. Student't test was used for statistical analysis. Treatment with GSH significantly reduced the severity of keratitis, conjunctivitis and palpebral edema compared to control group ($p<0.01$). In addition, on the 10th day after infection, all treated rabbits were completely cured. At the same time, in the control group, all eyes still showed countable lesions and five animals died between the 13th and the 16th day post infection, showing signs of neurological involvement. No toxicity was observed in uninfected eyes. These results show a significant therapeutic effect of GSH in HSV-1 induced keratitis and suggest the possibility that GSH modulates the neurotropism of the virus.