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THE RELATIVE ANTIVIRAL EFFICACY OF TWICE DAILY CIDOFOVIR VERSUS ACYCLOVIR AND TRIFLURIDINE IN THE HSV-1/NZ RABBIT KERATITIS MODEL ((Y.J. Gordon and E.G. Romanowski)) University of Pittsburgh and Medical Center, Pittsburgh, PA.

Purpose. Previously, we demonstrated that frequent topical dosing of 0.2% cidofovir (CDV) was effective in the treatment of experimental HSV-1 keratitis (Gordon et al, Cornea 1994;13:516-520). In the current study, we compared the efficacy of **twice daily** dosing of higher concentrations of CDV to trifluridine (Viroptic® [VIR]) and acyclovir (Zovirax® [ZOV]) in the HSV-1/NZ rabbit keratitis model. **Methods.** In a series of four experiments, 80 NZ rabbits were inoculated in both eyes with HSV-1 McKrae. Forty-eight hours after inoculation, the rabbits were randomly divided into 5 treatment groups: 1% CDV BID for 7 days; 0.5% CDV BID for 7d; 3% ZOV 5ID for 7d; 1% VIR 9ID for 3d, then QID for 4d; Control vehicle BID for 7d. All eyes were examined using a slit-lamp for keratitis on days 2, 3, 5, 7, 9, 11, and 14 P.I. and cultured for virus on days 1, 3, 5, 7, 9, 11, and 14 PI. **Results.**

	1% CDV	0.5% CDV	ZOV	VIR	Control
Mean Titer (D7)	$5.1\pm27\times10^{1}$*	$2.5\pm9.3\times10^{2}$*	$2.3\pm3.4\times10^{2}$*	$1.5\pm4.0\times10^{3}$*	$1.4\pm2.3\times10^{5}$
+ Eyes/Total	48/174*@	65/180*@	104/174*	136/176	127/178
Shedding (D)	4.1 ± 1.5*@	5.1 ± 0.9*@	8.2 ± 4.3	9.7 ± 3.9	9.6 ± 1.9
Keratitis (D7)	0*@	.03±.12*@	.29±.31*	.66±.46*	2.3±.49

*P<0.05 when compared to Control, @P<0.05 when compared to ZOV and VIR

Conclusion. On day 7, all antiviral treatments significantly reduced viral titers and keratitis scores compared to Control vehicle. Overall, topical CDV (1% & 0.5%) twice daily was significantly more effective than ZOV and VIR in reducing HSV-1 positive eyes, duration of HSV-1 shedding, and day 7 keratitis scores in the HSV-1/NZ rabbit keratitis model.

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Evaluation of Liposome-encapsulated Foscarnet in the Rabbit Eye.

F. Hoffmann[1,2], H. Gümbel[3], F. Stieneker[1], J. Kreuter[1], J. Cinatl[4], J. Cinatl, Jr.[2]

[1]Inst. of Pharm. Technology, [2]Inst. of Med. Virology, [3]Center of Ophthalmology, [4]Centre of Pediatrics, J.W. Goethe-University, Frankfurt/M., Germany

We determined the ocular pharmacokinetics and toxicity of foscarnet after intravitreal and eye drop application of liposome-encapsulated foscarnet. 66 New Zealand white rabbits were used for this study. For the intravitreal part foscarnet loaded liposomes with a negative surface charge were given to 21 rabbits into both eyes. As positive control 21 rabbits were treated with a single dose of 2.4 mg foscarnet in phosphate-buffered solution given into both eyes. Aqueous humor, vitreous, blood and retina were collected at 1, 3, 6, 12, 24, 48 and 72 hours. For the second part of the study positively charged liposomes with encapsulated foscarnet were applicated to 9 rabbits as eye drops. Another group of 9 rabbits served as positive controls. After 15, 60 and 120 min. aqueous humor, vitreous, blood and cornea were collected. Determination of foscarnet was carried out by high-performance liquid chromatography and electrochemical detection. After intravitreal application vitreous levels ranged from 448 to 3918 µM for liposome-encapsulated foscarnet and from 287 to 4012 µM for the free drug. Terminal elimination half-life in the vitreous could not be prolonged by liposome-encapsulation. Aqueous humor levels ranged from 93 to 216 µM for the liposome preparation and from 42 to 186 µM for the drug solution. Neither empty nor loaded liposomes with a negative surface charge caused retinal toxicity. No detectable foscarnet concentrations could be found after eye drop application of free or liposome-encapsulated foscarnet. Results showed that liposome-encapsulation of foscarnet did not enhance drug levels in the vitreous, while a moderate increase of foscarnet concentration in aqueous humor could be found.

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Inhibition of Human Cytomegalovirus by PMEA. X. Xiong, C. Flores, J.M. Cherrington, M.D. Fuller, D.B. Mendel, K. Moon, and M.S. Chen. Gilead Sciences Inc., Foster City, CA, U.S.A.

PMEA [9-[2-(phosphonomethoxy)ethyl]adenine] is an acyclic nucleotide analog with broad spectrum antiviral activity against retroviruses, hepadnaviruses, and herpesviruses including human cytomegalovirus (HCMV). Data from a Phase I/II clinical study of adefovir dipivoxil (the oral prodrug of PMEA) indicated that PMEA is active against HIV and suggested it also has anti-CMV activity. To elucidate the basis of this anti-CMV activity, we investigated the inhibition of HCMV DNA polymerase by PMEA diphosphate (PMEApp), the putative antiviral metabolite of PMEA. We also compared the metabolism of PMEA and cidofovir in CMV disease-relevant cell lines and addressd the *in vitro* antiviral activity of PMEA against CMV clinical isolates. PMEApp is a potent, competitive (with regard to dATP) inhibitor of HCMV DNA polymerase with a K_i value of 0.45±0.06 µM. The catalytic efficiencies for HCMV DNA polymerase-catalyzed incorporation of PMEApp, ganciclovir triphosphate, and cidofovir diphosphate (CDVpp) are 0.18, 0.036, and 0.024, respectively. PMEA and cidofovir are taken up effectively by several HCMV disease-relevant cell lines, and are also efficiently phosphorylated to the active antiviral metabolites PMEApp and CDVpp in all cell lines tested. PMEA is effective against laboratory CMV strains as well as CMV clinical isolates in cell culture-based assays. These data indicate that PMEA would inhibit HCMV DNA synthesis and are consistent with the anti-CMV activity observed in AIDS patients.

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Individual Pre-screening of Antiviral Substances Using CYTOMORPH-b

R. Klöcking[1], J. Wönne[2] and P. Wutzler[1]

[1]Institute for Antiviral Chemotherapy, Friedrich Schiller University of Jena, D-99089 Erfurt, Nordhäuser Str. 78; [2]Dr. Fritz Nerbe Nachf. J. Nerbe Partnership, D-21423 Winsen/Luhe, Daimlerstr. 3, Germany

Pre-screening of antiviral drugs in 96 well microtiter plates is occasionally affected by toxic metabolites spreading over the plate via gaseous phase. In order to avoid mutual influence of various test substances, we developed a pre-screening assay that enables individual dose finding for antiviral drugs and a separate incubation of uninfected and infected cells. For this purpose, 3x3 well microtest plates CYTOMORPH-b for maximally 9 substance concentrations were used. The special construction of these plates allows to apply phase contrast microscopy to the morphological evaluation of cytopathogenic effect and the use of an ELISA reader for spectrophotometric measurements (required for MTT and EZ4U assay, respectively). Besides antiviral pre-screening, CYTOMORPH-b is also suitable for the in-situ detection of apoptotic cell death by means of TdT assay. Examples are demonstrated for the individual prescreening of antiherpetic compounds and for the in-situ detection of apoptosis in HCMV-infected human lung fibroblasts.