102

Interspecies Scale-Up of Antiviral Nucleoside Pharmacokinetics. P. T. Simmons and F. D. Boudinot. Department of Pharmaceutics, University of Georgia College of Pharmacy, Athens, GA 30602.

Interspecies variation in drug disposition can often be largely explained by differences in species body weight. Therefore, it is possible to develop allometric relationships between pharmacokinetic parameters and species body weight. Interspecies scaling of pharmacokinetic data obtained from preclinical laboratory animal studies can provide reliable predictions of pharmacokinetic parameters and plasma drug concentrations in humans. The application of interspecies scale-up techniques was applied to 7 nucleoside analogs: AZT, ddC, ddI, 3TC, D4T, AzdU, and AzddMeC. Significant correlations between pharmacokinetic parameters (total clearance, renal clearance, non-renal clearance, and steady-state volume of distribution) from laboratory animal models (mouse, rat, cat, dog, woodchuck, minipig, and monkeys) were observed. Based on these correlations, accurate predictions of nucleoside pharmacokinetic parameters in humans were attained. Plasma nucleoside concentrations as a function of chronological time were markedly different between species. However, when chronological time was converted to pharmacokinetic (physiological) time, these profiles were superimposible. The allometric time-transformed nucleoside concentration versus time data were employed to develop unit dose, species independent characterizations of nucleoside disposition. Using these characterizations, accurate predictions of plasma nucleoside concentration versus chronological time profiles in humans based on various doses were generated. These results demonstrate the rational use of preclinical nucleoside pharmacokinetic data for the prediction of pharmacokinetic parameters and plasma nucleoside concentration versus time profiles in humans. These predictions are useful for the design amnd evaluation of clinical trials of promising antiviral nucleoside analgues.

103

Evaluation of a Live Attenuated Recombinant Virus RAV 9395 as an HSV-2 Vaccine in Guinea Pigs.
F.C. Spector*, E. R. Kern#, J. Palmer#, R. Kaiwar,*
R. R. Spaete*.
*Aviron, Mountain View, California,USA
#Univ. Alabama, Birmingham, Alabama, USA

Recombinant virus RAV 9395 was constructed by deleting both copies of the $\gamma_1$34.5 gene, and the UL55 and UL56 open reading frames from HSV-2 (G). Both copies of ORF P have also been deleted because they reside on the opposite strand of the viral DNA to the $\gamma_1$34.5 genes. The thymidine kinase (*tk*) gene is intact and functional, conferring acyclovir sensitivity to the recombinant RAV 9395 at levels comparable to wild type HSV-2 viruses. The potential use of RAV 9395 as an HSV-2 vaccine candidate was investigated by evaluating the ability of RAV 9395 to protect guinea pigs from severe disease by HSV-2(G) challenge. Our results show that RAV 9395 administered intramuscularly was effective at reducing both lesion development and severity in guinea pigs infected with HSV-2 (G) in a dose dependent manner with a single dose. Reactivation of RAV 9395 from latency by co-cultivation of explanted doral root ganglia was observed at a much reduced frequency relative to HSV-2(G). Immunization with RAV 9395 at doses of $1X10^5$ pfu and above may preclude the establishment of latency by the challenge virus. The guinea pig studies reported here offer encouragement for the development of an efficacious live attentuated HSV-2 vaccine.

104

Evaluation of Potential Treatments for Epstein-Barr Virus Infections in Man Using a Murine Gammaherpesvirus-68 Infection Model. Donald F. Smee, K.W. Bailey, and R.W. Sidwell. Inst. for Antiviral Research, Utah State Univ., Logan, UT 84322-5600.

Murine gammaherpesvirus-68 (MHV-68) is used as a model for studying Epstein-Barr virus (EBV) infections. Fourteen compounds known to inhibit EBV or other herpesviruses were evaluated in rhesus monkey kidney (MA-104) cells for inhibition of MHV-68. PMEA, acyclovir, ganciclovir, cyclobutylguanine, and ganciclovir phosphonate caused 50% inhibition of virus at 5-10 μM; PMEDAP, HPMPA, HPMPC, HPMPG, FIAC, and FMAU were more potent, inhibiting virus plaque formation at 1-2 μM. FIAU showed the greatest effect, inhibiting the virus at 0.1 μM. Foscarnet and acyclovir phosphonate were inhibitory at 100 and 45 μM, respectively. BALB/c mice immunosuppressed with cyclophosphamide were treated with acyclovir, FIAU, HPMPC, or placebo for 5 days starting 24 h after intranasal MHV-68 challenge. Acyclovir (70 mg/kg/day) and FIAU (5 mg/kg/day) treatments caused 1-2 day delays in death. HPMPC (5 mg/kg/day) treatments resulted in a 14 day delay in death, and 4 $\log_{10}$ less lung virus than the placebo group on day 9 of the infection. HPMPC appears to be an excellent candidate for treating EBV infections, particularly in immunocompromised patients.

105

Identification of an Antisense Oligonucleotide with *in vivo* Activity Against Human Papillomavirus. 1. *In vitro* evaluation of oligonucleotides. P.C. Roberts[1], B.L. Frank[1], S.E. Boldt[1], D.M. Walther[1], J.L. Wolfe[1], R.E. Kilkuskie[1], D.E. Szymkowski[2], I.M. Greenfield[2], V. Sullivan[2], J.S. Mills[2]. [1]Hybridon Inc, Worcester, MA, USA; [2]Roche Research Centre, Welwyn Garden City, Herts, UK.

Human papillomavirus (HPV) types 6 and 11 are associated primarily with condylomata acuminata, benign genital warts. The E1 gene product of these viruses is essential for their replication. Using *in vitro* RNase H assays and cell based HPV E1-luciferase fusion assays we identified several 20mer phosphorothioate (PS) antisense oligonucleotides with E1-specific antisense activity. One of these oligonucleotides, HPV-1 PS, which has an EC_{50} of 30 nM in cell based assays, was selected for further evaluation. The antisense activity of HPV-1 has been shown to be sequence specific, by introduction of mismatches and comparison to sense and scrambled oligonucleotide controls. Its antisense activity is RNase H-dependent; an all 2'-O-methyl RNA oligonucleotide being inactive in cell based assays. Furthermore, E1 mRNA levels in cells transfected with a full length E1 gene are reduced in the presence of HPV-1 PS. Varying the length of HPV-1 from an 18mer to a 30mer had no effect on its antisense activity; however, shorter oligonucleotides had reduced antisense activity proportional to their RNase H activity and T_m. HPV-1 hybrid oligonucleotides with 2'-O-methyl RNA moieties were found to retain good antisense activity and were resistant to nuclease digestion. Chimeric oligonucleotides, which have a mixed DNA backbone, had varied antisense activities, dependent on the number and location of the modified nucleotides. We have also identified self-stabilized oligonucleotides in which 3' end loops increase resistance to exonuclease digestion; these retain their antisense activity in cell based assays. Several different derivatives of HPV-1 have been selected for additional testing for activity against HPV type 11 in the Kreider murine renal xenograft model (see abstract by Lewis *et al.*).