Here, we report the design of dansyl-tagged CADA analogs as fluorescent derivatives of the lead compound in order to study the cellular kinetics. The dansyl analogs were tested for their antiviral and CD4 down-regulating activity. Importantly, down-regulation of the CD4 receptor expression by the CADA compounds did not result in increased cytotoxicity. The dansyl-labeled derivative KKD-016 proved to have similar biological properties as the lead compound CADA. The use of KKD-016 in flow cytometric studies with UV-excitation showed a time- and dose-dependent uptake and CD4 down-regulating activity of KKD-016 in MT-4 and $CD4^+$-transfected cells. In addition, confocal microscopy revealed the presence of small vesicles of the compound in the cytosol of the cell. Interestingly, a similar distribution of the CADA derivative was observed in CD4 positive and negative cells, indicating that the uptake of the CD4 down-modulators is not restricted to the presence of CD4 on the cell surface. Further studies are ongoing with KKD-016 in order to reveal the specific mechanism of action of this new class of HIV entry inhibitors.

doi:10.1016/j.antiviral.2007.01.109

Poster Session II: Herpesviruses, Poxviruses, Other Antivirals and Medicinal Chemistry

102

An Animal Model of HCMV Infection in SCID Mice

Fernando Bravo*, Rhonda Cardin, David Bernstein

Division of Infectious Diseases, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA

Animal models for the evaluation of new therapies against human cytomegalovirus (HCMV) are limited due to the species-specific replication of CMV. Several models utilizing human tissues implanted into SCID mcie have, however, been used but are labor intensive. As an alternative, we have used biodegradable gelatin matric (Gelfoam) imbedded with HCMV-infected human foreskin fibroblasts (HFF) implanted into SCID mice. After evaluation of several parameters, the following model was selected for antiviral evaluations. HCMV GFP+ virus, HV5.111 (Toledo strain), was used to infect HFFs at a MOI of 0.01. Infected cells were then seeded 24 h later onto Gelfoam strips. After a 24-h incubation period, the Gelfoam strips were implanted subcutaneously into SCID mice using a trocar needle. To evaluate the effects of time and duration of therapy, implanted mice ($N = 6$ mice/group) were treated with ganciclovir (GCV) at 50 mg/kg/dose administered IP twice daily from day 0 to 5 or from day 0 to 14 or from day 7 to 14 after implantation. Treatment with GCV from day 0 to 5 produced a marginally significant reduction in viral titer compared to untreated controls. However, extended treatment from day 0 to 14 resulted in a significant reduction in viral titers ($1.62 \pm 0.32 \log_{10}$ pfu/ml) versus the untreated control ($3.09 \pm 0.39 \log_{10}$ pfu/ml), $P < 0.0001$). Viral titers were also significantly reduced ($1.59 + 0.32 \log_{10}$ pfu/ml, $P < 0.0001$) in the group receiving delayed GCV treatment (from day 7 to 14 post implantation) reflecting improved drug delivery due to increased vascularization of the implant over time. To further validate the model, another antiviral, cidofovir (CDV), was administered IP at 25 mg/kg/day from day 7 to 14 after implantation. A significant reduction in titer ($1.56 \pm 0.40 \log_{10}$ pfu/ml, $P < 0.0001$) was also observed in the CDV treated group compared to the untreated control ($3.51 \pm 0.31 \log_{10}$ pfu/ml). These results indicate that the Gelfoam-HCMV SCID mouse model is a simpler and more convenient alternative for the in vivo evaluation of new antivirals against HCMV.

Acknowledgement: Supported by Contract NO1-AI-15438 from the Virology Branch, NIAID, NIH.

doi:10.1016/j.antiviral.2007.01.110

103

Use of CpG DNA in Co-administration with Cidofovir or Monoclonal Antibody as a Post Exposure Antiviral Therapy

Amanda Phelps[1,*], Lin Eastaugh[1], Art Kreig[2], Amanda Gates[1]

[1] Dstl, Biomedical Sciences, Porton Down, Salisbury, WILTS, SP4 0JQ, UK; [2] Coley Pharmaceutical Group Ltd., USA

There is a need to develop effective antiviral therapies against orthopoxviruses and Venezuelan Equine Encephalitis virus (VEEV). Vaccines exist that are effective against these viruses but there are complications associated with their use. We wished to investigate the use of synthetic CpG DNA (CpG) in co-administration with other antiviral therapies for the treatment of infection caused by these viruses. Co-administration with Cidofovir (CDV) may mitigate the nephrotoxic effects through the use of much lower doses and fewer treatments. Likewise, co-administration with monoclonal antibody (MAb) may actually provide a treatment for VEEV infection where one does not currently exist.

Adult Balb/c mice were challenged with 20–100 MLD_{50} VACV and treated 1 day post challenge. A dose range of CDV was given as a single treatment (i/p) or in combination with CpG (i/n), and a group of mice were treated with CpG alone. 100% protection was observed in mice treated with CDV, or CDV + CpG at doses of 1.5–3 mg/kg. Mice treated with 3 mg/kg CDV + CpG did not lose significant weight or show any severe clinical signs of disease in comparison to mice treated with CDV only and CpG only. Doses of CDV <3 mg/kg did not provide any statistical difference in the protection or severity of disease seen between treatment groups.

Adult Balb/c mice were challenged with approximately $10MLD_{50}$ VEEV by the aerosol route and treated at 2, 24 and 72 h post challenge. Mice were treated with MAb (i/p), CpG (i/n) or a combination of the two. Sixty percent protection was observed in mice treated with a combination of MAb + CpG administered 2 h post challenge. Only 10% protection was observed in mice treated with MAb alone. By 24 h post challenge protection had decreased to 20% and 0% respectively, with no survivors if treatment was delayed to 72 h post challenge.

The data presented suggests a potential role for synthetic CpG in the treatment of infection caused by these viruses and is an area of work still under investigation.

doi:10.1016/j.antiviral.2007.01.111

104

Efficacy of Oral CMX-001 Therapy Against Human Herpes Virus-6 Infections in SCID-hu Mice

Debra Quenelle [1,*], Mark Prichard [1], Shannon Daily [1], Deborah Collins [1], Terri Rice [1], George Painter [2], Alice Robertson [2], Earl Kern [1]

[1] Department of Pediatrics, University of Alabama School of Medicine, USA; [2] Chimerix, Inc., CA, USA

Human Herpes Virus type 6 (HHV-6) is an infection in infants or immunocompromised individuals which can lead to neurological sequelae. Childhood exanthema, *roseola infantum*, also known as 6th's disease, is caused by HHV-6B and some infants experience convulsions, encephalitis or encephalopathy. The virus is lymphotropic and capable of replication in SCID-hu thymus liver implants, models previously used for determination of antiviral efficacy against human immunodeficiency virus (HIV) or human cytomegalovirus (HCMV). Using these models, we have evaluated CMX-001 (HDP-cidofovir) against both the GS strain of HHV-6A and the Z29 strain of HHV-6B. Briefly, SCID mice were surgically implanted with fragments of human fetal thymus and liver tissue under the renal capsule. Following engraftment, HHV-6A or 6B was directly inoculated into the graft using approximately 5 $\log_{10}$ of virus. Mice were treated with either vehicle or CMX-001 at 10 mg/kg once daily for 12 days beginning 24 h post infection. Samples were obtained approximately one or two weeks post viral inoculation. Real-time PCR was used to determine genome copy number/g of tissue. Samples from vehicle treated mice on day 6 or 13 had 3.7 $\log_{10}$ copies/g tissue of HHV-6A compared to 2.9 and 2.6 $\log_{10}$ copies/g tissue of HHV-6A in CMX treated mice from day 6 or 13, respectively. Samples from vehicle treated mice on day 7 or 14 had 3.6 or 5.8 $\log_{10}$ copies/g tissue of HHV-6B compared to 2.8 and 3.06 $\log_{10}$ copies/g tissue of HHV-6B in CMX treated mice from day 7 or 14, respectively. This represents a significant reduction in viral replication ($p < 0.001$) by day 14. These results indicate that CMX-001, in addition to having potent activity against CMV in the SCID-hu mouse model, also has excellent activity against HHV-6. The results further suggest that CMX-001 should be evaluated for efficacy in a variety of human herpesvirus infections in the immunocompromised host.

doi:10.1016/j.antiviral.2007.01.112

105

Efficacy of Delayed Therapy Using Combinations of ST-246 with CMX-001 Against Systemic Cowpox Virus Infections in Mice

Debra Quenelle [1,*], Mark Prichard [1], Kathy Keith [1], Deborah Collins [1], Robert Jordan [2], Dennis Hruby [2], George Painter [3], Alice Robertson [3], Earl Kern [1]

[1] Department of Pediatrics, University of Alabama School of Medicine, USA; [2] Siga Technologies, Inc., USA; [3] Chimerix, Inc., USA

Previous studies have shown that either ST-246 or CMX-001 (HDP-cidofovir) are effective in preventing mortality of mice infected intranasally with cowpox virus (CV) or vaccinia virus (VV). While earlier studies paved the way for each potential antiviral compound to move into Phase I clinical trials, evaluation of efficacy using suboptimal doses of these two agents has not been reported previously. As with most infectious agents, the emergence of drug resistance or intentional genetic manipulation to create drug resistant variants by bioterrorists is possible. An orally available drug combination for treatment of orthopoxvirus infections could alleviate some of these concerns, particularly if delayed treatments are effective. In cell culture the combination of ST-246 and CMX-001 resulted in synergistic efficacy with no increase in toxicity. To determine if this combination would result in enhanced efficacy in an animal model, ST-246 was given once daily at 10, 3 or 1 mg/kg with or without CMX-001 to mice infected with CV. CMX-001 was given similarly once daily at 3, 1 or 0.3 mg/kg. Treatments were initiated 72 h or 6 days post infection with CV. ST-246 was given together with CMX-001 as a once daily oral gavage using 0.2 ml volumes for 5 days. ST-246 alone increased mean day to death (MDD) at 10, 3 or 1 mg/kg, but did not improve survival. CMX-001 alone increased survival at 3 mg/kg when given at +72 h, but only increased mean day to death when delayed for 6 days. When ST-246 was given with CMX-001 at 6 days post infection, protection from mortality was significantly enhanced over single drug therapy. Combinations of various doses of these two compounds did not appear to show any additive toxicity and resulted in a synergistic effect on survival. These results indicate that a combination of these two agents act synergistically in vitro and in vivo and should be considered for use in orthopoxvirus infections in humans.

doi:10.1016/j.antiviral.2007.01.113