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.

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

102

An Animal Model of HCMV Infection in SCID Mice

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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.

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103

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

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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.