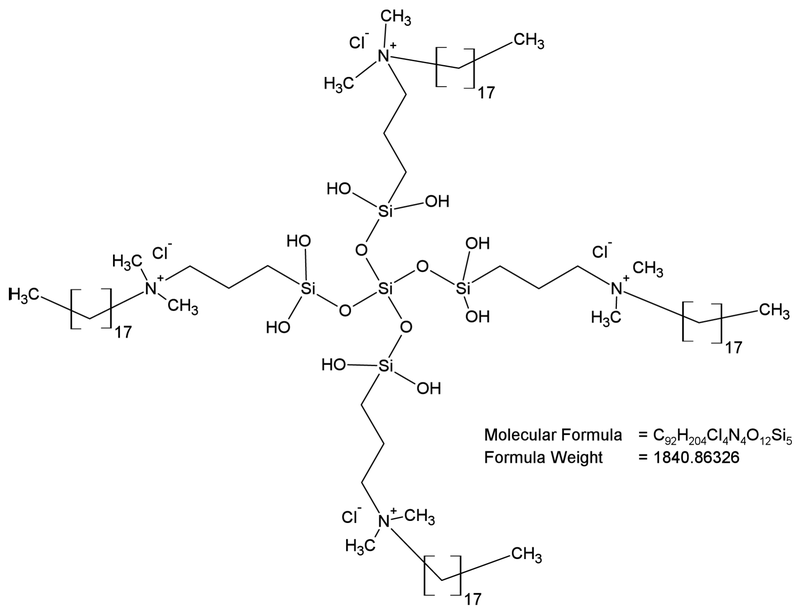
Anti-viral Activity of Quaternary Ammonium Silane (K21) Against HHV-6A and HHV-7

Nitish Gulve[1], Kirk Kimmerling[2], Gerhard Krueger[3], Dharam Ablashi[2,4], Bhupesh K. Prusty[1]

[1]Lehrstuhl für Mikrobiologie, Julius-Maximilians-Universität Würzburg, Germany.
[2]KHG fiteBac Technology, Marietta, USA.
[3]Department of Pathology and Laboratory Medicine, UT-Houston Medical School, Houston, USA.
[4]HHV-6 Foundation, Santa Barbara, USA.

Background

The quaternary ammonium silane (K21) is created through sol-gel chemistry, using an ethoxylated version of an organosilane quaternary ammonium compound and TetraEthyl Ortho Silicate (TEOS) as precursors. Hydrolysis and condensation of K21 with TEOS produces a 3-dimensional antimicrobial macromolecule with multiple arms of membrane rupturing potential (1). K21 was originally developed to be used in dental healthcare (i.e. in tooth cavities and for coating of implants). Antimicrobial assessment of K18 (the methacrylate version of the QAM) and K21 showed inhibited growth of several types of microorganisms including *E. coli*, *Staphylococcus aureus*, *Porphyromonas gingivalis* (2, 3) and *Chlamydia trachomatis* (Unpublished). As some of the Human herpesviruses including HSV-1, HHV-6A, HHV-6B, HHV-7, HCMV and EBV reside in the human oral cavities and are shed in the saliva to induce infection; we tested *in vitro* the effect of K21 on HSV-1, HHV-6 and HHV-7 infection.



Molecular structure of K21 molecule

Results

Cytotoxicity values of K21

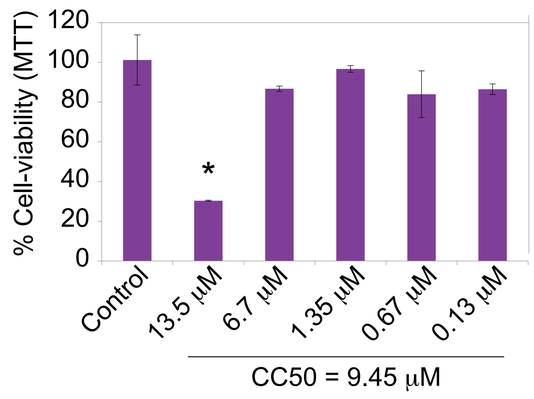


Figure 1. Cell viability (MTT) assay in primary human foreskin fibroblasts (HFFs) to determine cytotoxic dose (CC50) of K21. HFFs were seeded into 96-well plates (10^3 cells/well). At 24 h in culture with varying amounts of K21, cells were analyzed for viability by MTT assay. Results show the mean % cell viability relative to solvent control treated cells (Control) from two repeated experiments performed in triplicate. *p<0.05. CC50 value was calculated to be 9.45 µM from the calculated slope equation.

Effect of K21 on HHV-7

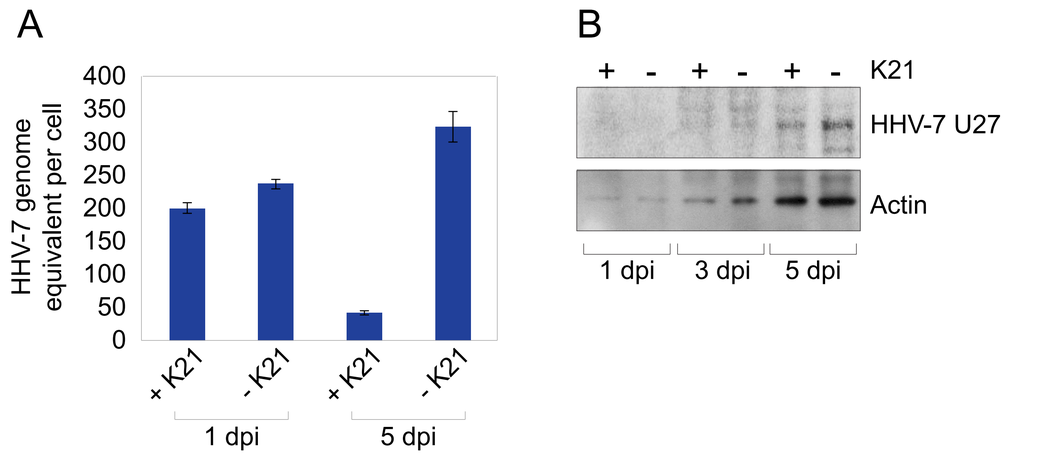


Figure 3. K21 inhibits HHV-7 infection. **(A)** Effect of K21 on HHV-7 growth was studied by immunoblotting. HHV-7 infected SupT-1 cells were mixed with uninfected SupT-1 cells at a ratio of 1:10 and were kept either in presence of K21 or solvent control for different time intervals. Total protein lysates were prepared and were analyzed for expression of HHV-7 late protein U27. Actin was used as loading control.

Effect of K21 on HHV-6A

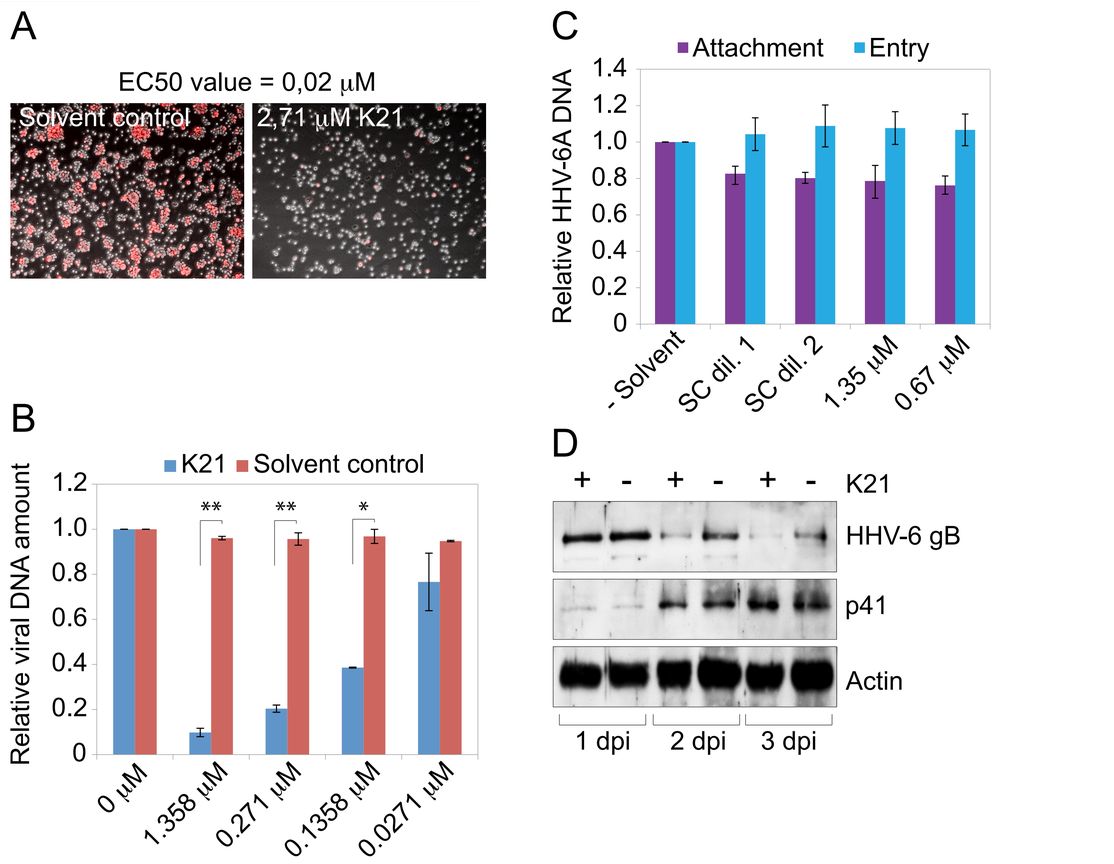


Figure 2. K21 inhibits HHV-6A infection. **(A)** HSB-2 cells were infected with HHV-6A that express mCherry protein in infected cells. Infected cells were treated with either solvent control or K21. At 72 h post infection, cells were imaged using epifluorescence microscope. **(B)** Total genomic DNA was extracted from a parallel set of experiment and HHV-6 DNA amount in infected cells were quantified by qPCR. **(C)** Effect of K21 on HHV-6A entry and attachment was studied by qPCR. SC, solvent control. **(D)** Effect of K21 on HHV-6 replication and growth was studied by immunoblotting.

Effect of K21 on HSV-1

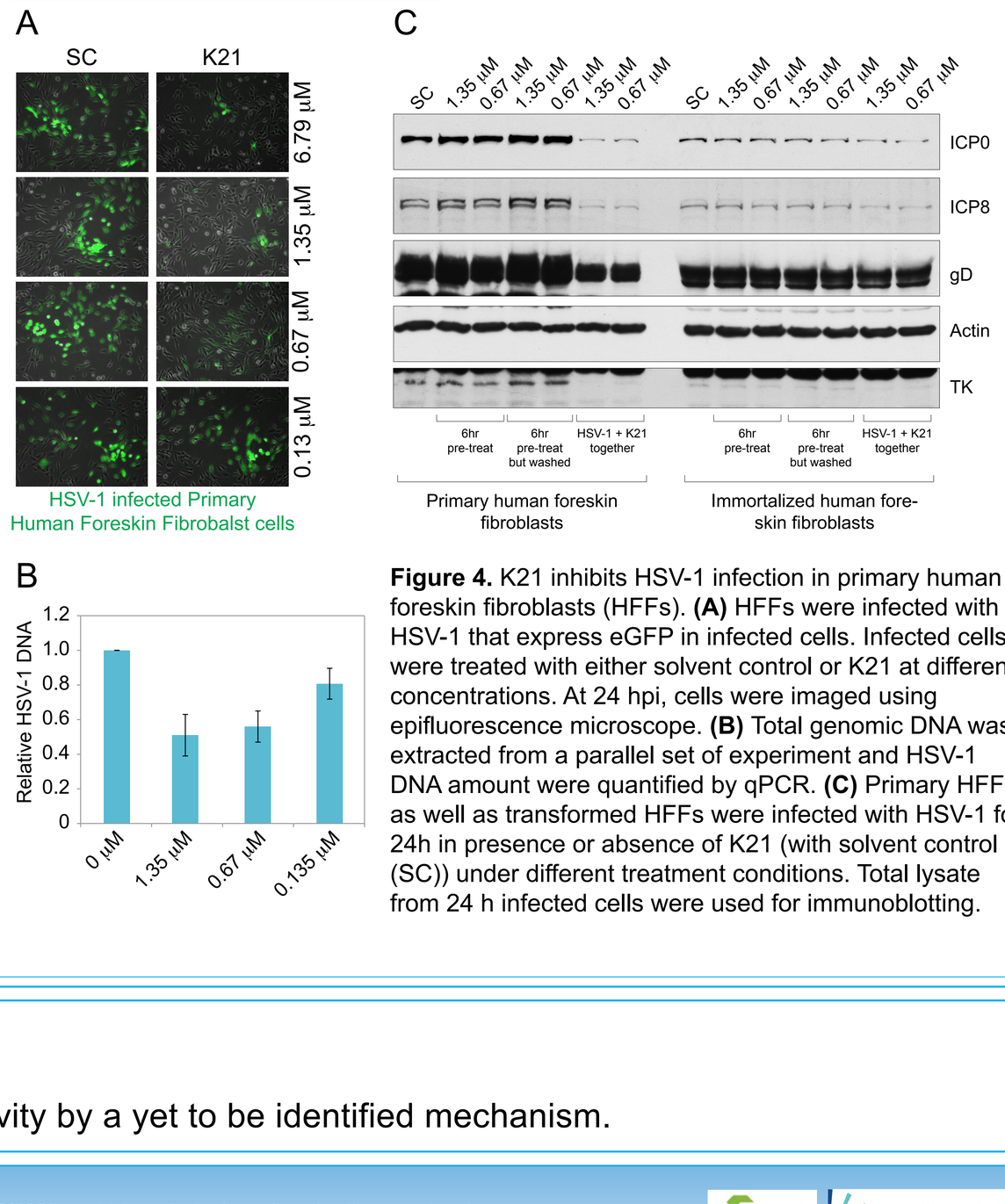


Figure 4. K21 inhibits HSV-1 infection in primary human foreskin fibroblasts (HFFs). **(A)** HFFs were infected with HSV-1 that express eGFP in infected cells. Infected cells were treated with either solvent control or K21 at different concentrations. At 24 hpi, cells were imaged using epifluorescence microscope. **(B)** Total genomic DNA was extracted from a parallel set of experiment and HSV-1 DNA amount were quantified by qPCR. **(C)** Primary HFFs as well as transformed HFFs were infected with HSV-1 for 24h in presence or absence of K21 (with solvent control (SC)) under different treatment conditions. Total lysate from 24 h infected cells were used for immunoblotting.

Conclusions

K21 inhibits growth and infection of HSV-1, HHV-6A and HHV-7 activity by a yet to be identified mechanism.

References:

1. Gong S-q, Epasinghe J, Rueggeberg FA, Niu L-n, Mettenberg D, Yiu CKY, Blizzard JD, Wu CD, Drisko CL, Pashley DH, Tay FR. An ORMOSIL-containing orthodontic acrylic resin with concomitant improvements in antimicrobial and fracture toughness properties. PLOS One 2012a;7(8):e42355.
2. Gong S-q, Niu L-n, Kemp LK, Yiu CKY, Rhou H, Qi Y-p, Blizzard JD, Nikonov S, Brackett MG, Messer RLW, Wu CD, Mao J, Brister LB, Rueggeberg FA, Arola DD, Pashley DH, Tay FR. Quaternary ammonium silane-functionalized, methacrylate resin composition with antimicrobial properties and self-repair potential. Acta Biomaterialia 2012b;8:3270-3282.
3. Meghil MM, Rueggeberg FA, El-Awady A, Miles B, Tay FR, Pashley DH, Cutler CW. Novel antimicrobial coating of surgical sutures and dental floss with activity against gram-negative and gram-positive bacterial pathogens. J Periodontol (in review, Sept. 2014).

Graduate School Life Sciences
VolkswagenStiftung