A novel antiviral compound inhibits diverse viral infections

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Abstract: We evaluated a novel compound, ML:8, for its ability to inhibit a range of viral infections in vitro. ML:8 treatment inhibited Epstein-Barr virus, measles virus and herpes simplex virus (HSV-1), together with Ebola, Lassa and vesicular stomatitis virus pseudoparticles, in a dose- and time-dependent manner. A 3D human skin equivalent model, Labskin, was used to model HSV-1 and Ebola virus infection in a physiologically relevant context. Labskin supported high levels of HSV-1 and Ebola pseudovirus infection which was completely inhibited using 3% ML:8. Inhibition of viral infection by ML:8 occurred without detectable cellular toxicity. ML:8 had no effect on norovirus, a non-enveloped virus, indicating that its mechanism of action may be via disruption of the viral envelope. We have identified a novel antiviral compound based on an emulsion of free fatty acids that may have applications for the prevention and treatment of a diverse range of enveloped viruses.

Background

1) ML:8 is a novel antimicrobial compound based on a fine emulsion of free fatty acids. Free fatty acids in milk are thought to have antiviral properties through interfering with viral envelope integrity1-3; therefore we evaluated the ability of ML:8 to inhibit a range of enveloped and non-enveloped viruses.

2) ML:8 is effective at pH 5.51, making it suitable for use on skin and mucous membranes. We therefore examined viral infections of skin, tonsil and airways using a range of physiologically relevant models.

3) A 3D human skin construct, Labskin2, was selected as a model for HSV-1 infection (‘cold sores’) and Ebola pseudovirus infection. Infection of primary tonsil epithelial cells was used as a model of glandular fever.

4) We evaluated the direct effects of ML:8 treatment on a range of enveloped viruses including Epstein-Barr virus (EBV), herpes simplex virus (HSV-1) and measles virus together with viral pseudotyped viruses including Ebola, Lassa and vesicular stomatitis viruses. Norovirus, a non-enveloped virus, was also evaluated.

Methods

1. Culture systems and selected viruses

Generation of viral pseudotypes. Plasmids containing the envelope glycoproteins from Ebola, Lassa or vesicular stomatitis virus (VSV) were co-transfected with the replication machinery from HSV-1 virus into 293T cells. Viral pseudotypes, bearing a luciferase reporter gene and capable of one round of replication, were harvested and used to infect target cells.

Generation of full length infectious viruses. Herpes simplex virus-1 (strain I7) was cultured and titred in Vero cells. Measles virus (strain IC323) and Epstein-Barr virus (strain strain 2089) were generated in 293T cells. Labskin was used to model HSV-1 and Ebola pseudovirus infections of human skin. Primary cultures of human tonsil epithelial cells were used to model EBV infection of the tonsil (glandular fever). A549 lung epithelial cells were used to model measles virus infection of the airway and Labskin and VSV infections of epithelial cells.

2. Antiviral assays using ML:8

Figure 1. Herpes simplex virus (HSV-1; 100PFU/mL) was treated with ML:8 at concentrations from 3% to 0.5%, and infectious titre quantified on Vero cells. 3% ML:8 fully inhibited HSV-1 infection, and neutralization was concentration dependent.

Figure 2. HSV-1 infected the basolateral side (dexamethasone) of the Labskin model following incubation for 72 hours. Labskin was infected with HSV-1 and treated with either 3% ML:8 or mock buffer. Cells were fixed and stained with an antibody to detect HSV-1. Nuclei were visualised with DAPI (virus). ML:8 reduced HSV-1 to undetectable levels in the Labskin human skin model.

Figure 3 (A) Primary tonsil epithelial cells were isolated as previously described. Cells were infected with a QFP-expressing isolate of Epstein-Barr virus (EBV). Virus was treated with the indicated concentrations of ML:8 or pH 5.5 control buffer for 2 minutes. Virus was then added to tonsil epithelial cells at an MOI of 10. After 72 hours, infected cells were enumerated. (B) Measles virus was treated with the indicated concentrations of ML:8, or pH 5.5 control buffer, for 2 minutes. Virus was then added to A549 lung epithelial cells (TCID50 4.5 x 10^4/mL) for 72 hours, and infected cells enumerated. ML:8 completely inhibited both viruses at a concentration of 3%. Furthermore, inhibition was concentration dependent.

Figure 5 (A) Labskin was wounded using a scalpel to create a small break in the keratinized epithelium, or left unwounded. Ebola pseudovirus was added to the epithelium for the indicated times, and then removed and washed five times with sterile PBS. After 72 hours, cells were lysed and luciferase activity quantified. Ebola pseudovirus infected Labskin in a time-dependent manner following apical infection (virus exposed to the apical sidewall). However, when the basolateral side of Labskin (‘dermis’), was exposed to virus, no infection occurred, suggesting that Ebola infects the epidermal layer of Labskin. (B) ML:8 abolished Ebola pseudovirus infection of Labskin. Virus was exposed to the indicated concentrations of ML:8 for 2 minutes, and then the epidermal side of non-wounded Labskin was infected. Infection is presented relative to control without a viral envelope (no envelope). ML:8 completely inhibited Ebola pseudovirus entry to Labskin and inhibition was concentration dependent.

Conclusions

1) ML:8 inhibited a range of enveloped viruses including HSV-1, Epstein-Barr and measles virus infection, together with Ebola, Lassa and VSV pseudoviruses. ML:8 had no effect on norovirus, a non-enveloped virus [data not shown], indicating that its mechanism of action may be via disruption of the viral envelope.

2) Inhibition of infection occurred in the absence of cellular toxicity, measured using MTT assays.

3) Herpes simplex virus and Ebola pseudovirus infect a human 3D skin model, Labskin. Ebola pseudovirus infects both wounded and non-wounded Labskin via the apical (epidermal) side, which may indicate that Ebola virus is capable of direct infection of skin.

4) ML:8 also inhibits Lassa and VSV pseudoviruses [data not shown], similar to Ebola pseudovirus.

1) These results demonstrate for the first time that ML:8, a novel compound based on an emulsion of free fatty acids, inhibits a range of enveloped viruses in physiologically relevant in vitro models. These findings provide a rationale for future clinical studies.