

Services Laboratories, Ames, IA, USA), which was initially passaged 5 times in Vero cells, was used to infect the MK-DIECs. After adsorption for 1 h, the cells were cultured in serum-free Dulbecco modified Eagle medium supplemented with 0.02% yeast extract, 0.3% tryptose phosphate broth, and 1% penicillin/streptomycin (infection medium). To examine the requirement of trypsin (Sigma, St. Louis, MO, USA) for PEDV replication in MK-DIECs, we added trypsin (0, 2.5, 5, or 10 µg/mL) to the infection medium.

We also cultured MK-DIECs in 96-well plates and similarly infected them with PEDV for the detection of PEDV nucleoprotein (NP) by IFA using fluorescein isothiocyanate-labeled mouse PEDV NP monoclonal antibody (SD-1F; Medgene Labs, Brookings, SD, USA). At 12, 24, and 36 h after infection, released virus in infected cells was quantified by virus titration in Vero cells by inoculating 10-fold serial dilutions. After 24 h, viral NP was detected by IFA staining. The virus titer was calculated according to the Reed–Muench method and expressed as the 50% tissue culture infectious dose/mL.

We detected PEDV NP in MK-DIECs 24 hours after infection in medium with and without trypsin (data not shown). However, the numbers of cells positive for PEDV NP was larger in cells cultured with trypsin (2.5 µg/mL and 5 µg/mL) than without trypsin (Figure, panel A). PEDV also induced cytopathic effects (CPEs) in these cells, which were characterized by rounding of cells, syncytium formation, and cell detachment. The CPEs were more pronounced in cells infected with added trypsin; as little as 2.5 µg/mL of trypsin in infection medium was sufficient to induce substantial CPEs in infected cells (Figure, panel B). No signs of CPEs were observed in uninfected control cells, and cells did not display any trypsin-mediated toxicity. The virus titers were detectable in PEDV-infected cells 12 h after infection. The titers further increased at 24 h, reaching a peak at 36 h after infection. Infected cells in infection medium with 10 µg/mL added trypsin had the highest titers (online Technical Appendix Figure 2).

Coronaviruses are RNA viruses that are prone to high levels of mutation resulting in novel reassortants. Birds and bats are considered reservoirs of coronaviruses. However, reservoirs of PEDV are not yet known. In conclusion, we have demonstrated that PEDV replicates in MK-DIECs. Availability of a cell line of intestinal origin that supports PEDV replication may be of value for studying mechanisms of virus–cell interactions and for developing live attenuated and killed vaccines.

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Lack of Effect of Lamivudine on Ebola Virus Replication

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To the Editor: The unprecedented number of Ebola virus disease (EVD) cases in western Africa has compelled the world to consider experimental and off-label therapeutics to mitigate the current outbreak. For clinicians, approved drugs are an attractive solution because of known safety profiles and availability.

Oral lamivudine (GlaxoSmithKline, Brentford, UK), a US Food and Drug Administration–approved anti-HIV drug, has been suggested as a possible antiviral agent against Ebola virus (EBOV). In September 2014, a Liberian physician, Dr. Gorbée Logan, reported positive results while treating EVD with lamivudine (*1*). Thirteen of 15 patients treated with lamivudine survived presumed EVD and were declared virus free. Clinical confirmation of EVD in these cases remains to be verified.

Our laboratory had previously assessed this antiretroviral compound in drug screens against EBOV and observed no discernable antiviral activity. However, given

Table. Inhibitory effects of test compounds on Ebola virus replication*

Virus variant or subtype	Cell type	Lamivudine EC ₅₀ , μmol/L†	Toremifene EC ₅₀ ± SD, μmol/L‡
EBOV/Kik	Vero E6	>80	5.7 ± 0.7
EBOV/Kik	Vero E6	>320	12.0 ± 1.0
EBOV/Gue	Vero E6	>320	≈8
EBOV/Kik	HepG2	>80	1.6 ± 0.1
EBOV/Kik	HepG2	>320	5.5 ± 0.1
EBOV/Kik	Macrophages	>320	≈25
EBOV/Kik	Macrophages	>320	18.3 ± 0.8
HIV-1	Macrophages	0.002‡	ND
HIV-1	Monocytes	0.69‡	ND
HIV-1 (multiple subtypes)	PBMC	0.002–2.5‡	ND
HBV	HepG2 (2.2.15)	0.002§	ND

*EBOV, Ebola virus; EC₅₀, 50% effective concentration; BMC, peripheral blood mononuclear cells; ND, not done; HBV, hepatitis B virus.

†EC₅₀s were determined by using an EBOV ELISA with antibodies against glycoprotein or viral matrix protein as described (3).

‡Data from Schinazi (4).

§Data from Kruining et al. (5).

the recent testimonials regarding lamivudine effectiveness in treating EBOV-infected patients in Africa, we conducted additional studies to determine whether our previous assertion that lamivudine lacked any direct antiviral activity was correct.

Lamivudine is a nucleoside analog reverse transcription inhibitor of HIV and hepatitis B virus that acts as a synthetic cytidine analog. Incorporation of the active triphosphate form into viral DNA results in chain termination. Studies have demonstrated that lamivudine is a weak inhibitor of mammalian α , β , and γ DNA polymerases (2). Lamivudine would not be expected to inhibit the replication of a negative-strand RNA virus.

The activity of lamivudine against EBOV infection was evaluated in a cell-based ELISA with 1995 isolate EBOV *H. sapiens-tc/COD/1995/Kikwit* (EBOV/Kik) (3). Three cell lines were tested: Vero E6 (African green monkey kidney, ATCC CRL-1586), Hep G2 (human hepatoma, ATCC HB-8065), and human monocyte-derived macrophages. Macrophages were generated by treating CD14⁺ cells for 7 days with macrophage colony-stimulating factor and conditioned medium. Cells were treated with compounds in 3-, 4-, or 8- point dose response curves with 2-fold dilutions starting at 80 μmol/L or 320 μmol/L oral lamivudine. Toremifene (T7204–5MG; Sigma-Aldrich, St. Louis, MO, USA) was used as a positive control for activity against EBOV and tested at 2-fold dilutions starting at 25 μmol/L. One hour after drug addition, the cells were infected at a multiplicity of infection of 0.5 or 1 with EBOV/Kik. Experiments were run on duplicate plates or the entire experiment was run on 2 separate days. At 48 hours after infection, cells were formalin-fixed and stained with a primary antibody against EBOV (antibodies against viral matrix protein or glycoprotein) and a secondary antibody (Alexa-488 or horseradish peroxidase).

No direct antiviral effect for lamivudine was observed at concentrations \leq 320 μmol/L in Vero E6 cells (Table). Because optimal efficacy of the drug requires phosphorylation,

lack of activity may be caused by poor phosphorylation in Vero E6 cells (6). Therefore, we also assessed HepG2 cells and primary human monocyte-derived macrophages sensitive to EBOV infection. Toremifene was included as a positive control. Toremifene is a US Food and Drug Administration–approved drug that was reported to have direct antiviral activity in cell culture and to protect mice infected with mouse-adapted EBOV (3). As expected, toremifene inhibited EBOV at low micromolar concentrations (Table).

Finally, we assessed the antiviral activity of the compounds against a recent isolate prototype from the current outbreak, EBOV *H. sapiens-tc/GIN/2014/Guékédou-C05* (EBOV/Gue) to test whether inhibition of EBOV/Gue by lamivudine was different from that of the reference Kikwit strain. In contrast to a known active compound (toremifene), lamivudine showed no direct antiviral activity.

The current data suggest that lamivudine does not directly inhibit EBOV RNA polymerase or replication of the virus. Systemic and off-target effects, while not previously described, might be possible. To address this possibility, we plan to assess lamivudine in the mouse model of EVD and will report these findings when available. However, on the basis of these in vitro tests, there is no foundation for recommending lamivudine for treatment of EVD in human patients.

G.G.O. was named in patent #8,475,804 assigned to the US Army on approved drugs for use for filoviruses.

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Correction: Vol. 21, No. 2

The abbreviation mL was inadvertently used in the place of μ L in paragraphs 2 and 4 of the print edition of the article Potential Sexual Transmission of Zika Virus

(D. Musso et al.) The article is correct online (http://wwwnc.cdc.gov/eid/article/21/2/14-1363_article.htm)

The image is a screenshot of the CDC Emerging Infectious Diseases (EID) website. At the top, there is a search bar with a 'SEARCH' button and a 'EID ONLY' checkbox. Below the search bar is the CDC logo and the text 'Centers for Disease Control and Prevention CDC 24/7: Saving Lives. Protecting People.™'. The main heading is 'EMERGING INFECTIOUS DISEASES®'. On the left side, there is a navigation menu with options like 'EID journal', 'August 2014', 'Past Issues', 'About the Journal', 'Manuscript Submission', 'Subscribe', 'Ahead of Print / In Press', 'Author Resource Center', 'Medscape CME', 'More Content', 'Social Media', and 'Contact Us'. At the bottom of the menu is a 'SUBMIT MANUSCRIPT' button. The main content area features 'Expedited Ahead-of-Print Articles' with two articles listed: 'Respiratory Viruses and Bacteria among Pilgrims during the 2013 Hajj' by S. Benkouiten et al. and 'Human Influenza A(H7N9) Virus Infection Associated with Poultry Farm, Northeastern China' by M. Fan et al. Below the articles, there is a section for 'Volume 20, Number 8—August 2014' with a 'PDF Version' link (PDF - 7.58 MB - 167 pages) and a 'Podcasts' link. The 'Last Issue - Volume 20, Number 7—July 2014' is also mentioned. At the bottom of the screenshot, there is a large text overlay: 'SEARCH PAST ISSUES OF EID AT WWWNC.CDC.GOV/EID'.