Antiviral potency of an extract from Nerium oleander

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INTRODUCTION
Targeting of multiple viral replicative activities with a single therapeutic is a highly coveted goal. Recent approaches using chimeric antibodies with dual specificities have demonstrated therapeutic efficacy in murine models against EBOV and SUDV, with extended efficacy against SUDV, TAFIV, and RESTV in vitro. The breadth of efficacy, however, is still constrained by genus as efficacy against Ebooviruses (EBOV, MARV) appears to be genus specific. PBI-05204 is a cardiac glycoside-containing extract from Nerium oleander that has demonstrated antiviral efficacy and is a cancer therapeutic in clinical trials. PBI-05204 and oleandrin, a cardiac glycoside that is one of the therapeutic agents in the PBI-05204 extract, are assessed for antiviral efficacy.

OBJECTIVES
Assess broad spectrum potency of extract from Nerium oleander.

BACKGROUND
Cardiac glycosides are used therapeutically to correct irregular heart rhythms and congestive heart failure. The cardiac glycoside oleandrin, as well as an oleandrin-containing extract of Nerium oleander known as PBI-05204, have also been shown to have antitumor potential in vitro and in vivo. Furthermore, PBI-05204 has been used in phase I and II clinical trials against solid tumors and does not result in any significant cardiotoxicity. Interestingly, oleandrin contained within the PBI-05204 H. oleander extract has been shown to cross the blood-brain barrier in mouse models. Recent studies have uncovered potential roles for cardiac glycosides as antiviral drugs against HIV-1, HSV-1, and HTLV-1 through a variety of mechanisms. Cardiac glycosides disrupt cellular Na+-K+-ATPase, an enzyme that is essential for maintaining the intracellular balance of calcium in smooth muscle cells in the heart by regulating the flow of sodium and potassium ions. Depressed Na+-K+-ATPase activity therefore leads to dysregulated calcium channel function. The indirect inhibition of calcium regulation by cardiac glycosides suggests that these drugs may also be effective against farnesasas calcium channel blockers have demonstrated efficacy against filoviruses. Calcium channel blockers have been demonstrated to mediate their antiviral effect by inhibiting new virus particle formation.

MATERIALS AND METHODS
A defined extract from Nerium oleander and purified oleandrin were used to pretreat Vero cells prior to and post-infection with MARV and BVDV. An immunofluorescence-based assay was used to determine antiviral efficacy 48hr post-infection. For passaging experiments, Vero cells were infected in the presence of PBI-05204 or oleandrin, supernatant was collected 48hr later. The supernatants were then assayed for the presence of infectious virus. An EBOV titration was used to assess viral transcription and replication in the presence of PBI-05204 or oleandrin.

RESULTS
PBI-05204 and oleandrin fully inhibited MARV and EBOV infection in Vero cells. No infectious progeny virus was recovered from supernatants of cells infected with EBOV or MARV when treated with PBI-05204 or oleandrin. Neither virus transcription or replication were inhibited by treatment with PBI-05204 or oleandrin, indicating the inhibitory effect of oleandrin does appear to be linked to viral polyenase function. Preclinical results also indicate PBI-05204 and oleandrin have antiviral activity against other enveloped viruses, demonstrating a broad antiviral profile.

CONCLUSION
The broad spectrum efficacy we’ve presented may be especially critical as certain therapeutic elements within the PBI-05204 botanical drug can be found to accumulate in the CNS, which is essential for viruses that have demonstrated neuropathic effects.

DISCUSSION
• Oleandrin and PBI-05204 exhibit antiviral efficacy that appears consistent with previous studies and do not prevent budding of new virions.
• PBI-05204 has very similar activity profile to oleandrin, suggesting oleandrin is active compound in PBI-05204 extract.
• Ability of oleandrin to cross the blood-brain barrier and efficacy against neurotropic alphaviruses suggests PBI-05204 has therapeutic potential.

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REFERENCES

FIGURE 1. Cardiac glycosides inhibit EBOV and MARV in vitro. Vero E6 cells were treated with oleandrin, digoxin or PBI-05204, an oleandrin-containing plant extract, for 1hr pre-infection then infected with EBOV/IA (A), MARV/CUN (B), or MARV/3166 (C) in the presence or absence of compounds. After 1hr, incubum and compounds were removed and fresh medium added to cells. 48h later infected and immunostained to detect cells infected with EBOV or MARV. Infected cells were enumerated using an Operetta. C) EBOV infected cells were treated with compound for 2hr or 24hr. ATP levels were measured by CellTitre-Glo as an indicator of cell viability.

FIGURE 2. Cardiac glycosides inhibit virus when treated shortly after infection. Vero E6 cells were infected with EBOV (A) or MARV (B). After 1hr, virus was removed and oleandrin or PBI-05204 was added immediately to the cells for 24h (C) after which compounds were discarded and cells were returned to medium. Alternatively, compounds were added 24h post-infection and incubated for 24hr (D). By 48hr total, infected cells were analysed as in Figure 1.

FIGURE 3. Cardiac glycosides do not inhibit EBOV transcription or replication. An EBOV monoglic was utilized to determine the efficacy of oleandrin or PBI-05204 in inhibiting virus transcription (A) or replication (B) BSR/T7 cells were transfected with DNA plasmids encoding for TF polymerase-driven expression of EBOV L, NP, VP30, VP50 (for transcription) and a luciferase reporter in adenoviral orientation. Plasmids were a kind gift from E. Mullighan.

FIGURE 4. Treatment with cardiac glycosides inhibits the production of infectious progeny. Vero E6 cells were infected with EBOV or MARV in the presence of oleandrin or PBI-05204. After 1hr, virus was removed, fresh medium was added and cells were incubated for 48h. Supernatant from infected cell cultures was passed onto fresh Vero E6 cells, incubated for 1hr, then discarded (as depicted in A). Cells containing passaged supernatant were incubated for 48h. Cells infected with EBOV (B) or MARV (C) were counted as described previously. Control infection rates were 65% for EBOV and 67% for MARV.

FIGURE 5. Cardiac glycosides are effective against alphavirus infection. Vero E6 cells were infected with Venezuelan equine encephalitis virus (A, MOI=0.1) or Western equine encephalitis virus (B, MOI=0.1) in the presence of oleandrin or PBI-05204. By 24hr, infected cells were detected as in Figure 1.