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γ-Carboline Derivatives as Potent and Selective Inhibitors of Bovine Viral Diarrhea Virus (BVDV) Replication

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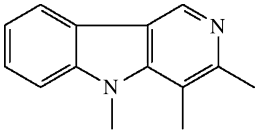
Novel γ-carboline derivatives were examined for their inhibitory effect on bovine viral diarrhea virus (BVDV) replication in cell cultures, and some compounds were found to be active against the virus. Among them, 3,4,5-trimethyl-γ-carboline (SK3M4M5M) was found to be the most active against BVDV in MDBK cells, and its EC_{50} and CC_{50} were 0.017 ± 0.005 and 7.4 ± 0.9 μM in virus (Nose strain) and mock-infected cells, respectively. The compound also suppressed viral RNA synthesis in a dose-dependent fashion. Studies on the mechanism of action revealed that SK3M4M5M did not interfere with viral entry. It has been reported that a single cycle of BVDV replication takes 13 h on average and that gradual increase of intracellular viral RNA is noted at 6–8 h after virus infection (Paeshuyse et al., J. Virol., 2006). In our time-of-addition experiment, SK3M4M5M lost its antiviral activity, when added after 8 h from viral infection. When the selected γ-carboline derivatives, including SK3M4M5M, were examined for their inhibitory effect on three strains that were resistant to BVDV RNA-dependent RNA polymerase inhibitors (AG110, LZ37, and BPIP), the strains showed cross-resistance to the γ-carboline derivatives. These results suggest that the γ-carboline derivatives target viral RNA polymerase. Although SK3M4M5M was not inhibitory to HCV in a RNA replicon cell system, it displayed modest anti-HCV activity (EC_{50} = 5.1 μM, CC_{50} = 41.3 μM) in cell-free JFH-1 infection assay.



SK3M4M5M

doi:10.1016/j.antiviral.2010.02.359

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Combination of Peramivir and Rimantadine Demonstrate Synergistic Interaction in Influenza a Mouse Model

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Background: In the event of an influenza outbreak, two classes of antivirals, the neuraminidase (NA) inhibitors and M2 ion channel blockers, may provide valuable benefit. These anti-influenza drugs act by different mechanisms and stages of the virus replication cycle. Efficacy of combination of intramuscular (IM) administered NA inhibitor, peramivir (P), and orally administered M2 ion channel blocker, rimantadine (R), was evaluated in cell culture and mice infected with influenza A/Victoria/3/75 (H3N2) virus.

Methods: Mice were infected with mouse adapted influenza A/H3N2/Victoria/3/75. Peramivir was administered by IM injection, qd and rimantadine was administered orally, bid. Five day dosing began 1 h prior to infection with virus. Each infected, drug treated group contained 9–10 mice and the saline treated group contained 15 mice. For in vitro studies, neutral red dye uptake assay was used.

Table 1
Effect of combinations of peramivir and rimantadine on weight loss in influenza A (H3N2) infected mice.

Compound (mg/kg/d)	Mean weight change at day 10 (g) ± SEM			
	Peramivir (P)			
Rimantadine (R)	0.0	0.3	1.0	3.0
0.0	−5.19 ± 0.16	−2.6 ± 0.75[a]	−4.3 ± 0.42[a]	−3.55 ± 0.35[a]
5.0	−3.43 ± 0.55[a]	−1.97 ± 0.47[a,b]	−1.69 ± 0.63[a,c]	−1.31 ± 0.34[a,c]
10.0	−2.1 ± 0.37[a]	−1.25 ± 0.55[a,d]	−0.69 ± 0.25[a,c]	0.05 ± 0.22[a,c]
30.0	−1.64 ± 0.54[a]	−1.52 ± 0.42[a]	−0.41 ± 0.22[a,e]	0.25 ± 0.14[a,c]

[a] $p < 0.05$ vs. vehicle, infected.
[b] $p < 0.05$ vs.0P/5R.
[c] $p < 0.05$ vs. either compound used alone.
[d] $p < 0.05$ vs. 0.3P/0R.
[e] $p < 0.05$ vs. 1P/0R.

Results: Weight loss is a sensitive indicator of response to virus infection. Significant decreases in weight loss were noted in combination treated groups vs. single agent and vehicle treated groups (Table 1). Three-dimensional analyses of weight loss for combinations of 1 mg/kg/d peramivir with 5, 10 and 30 mg/kg/d rimantadine and 3 mg/kg/d peramivir with 5, 10 and 30 mg/kg/d rimantadine demonstrated synergistic effects. In vitro combination studies with peramivir and rimantadine showed mainly additive and some synergistic effects.

Conclusion: These data support investigation of the combination of peramivir and rimantadine for the treatment of influenza in the clinic.

doi:10.1016/j.antiviral.2010.02.360

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A Single Intramuscular Injection of Peramivir Demonstrates Anti-influenza Activity Against Recently Isolated Pandemic Flu Virus H1N1 (A/CA/04/2009)

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Background: Peramivir is a potent and selective inhibitor of influenza neuraminidase. Here we demonstrate the efficacy of a single intramuscular (IM) injection of peramivir in mice infected with recently isolated pandemic flu virus H1N1 A/CA/04/09 (swine origin).

Methods: In the treatment model peramivir was administered as a single IM injection at 50 mg/kg dose and oseltamivir was given orally at 10 mg/kg/d bid for 5 days. In the prophylaxis model peramivir was given as a single IM injection. Each infected, drug treated group contained 9–10 mice and the saline treated groups contained 10–15 mice.

Results: Peramivir potently inhibits the neuraminidase enzyme N1 from H1N1 (A/CA/04/09) *in vitro* with an IC_{50} of 0.43 ± 0.07 nM ($n = 2$). A single IM injection of peramivir, given 1 h prior to infection (prophylaxis model), significantly reduced weight loss and mortality in mice infected with pandemic influenza A/H1N1 virus (Table 1). In the therapeutic treatment model, peramivir given 24, 48 and 72 h after infection as a single IM injection at 50 mg/kg dose, showed significant protection against lethality. There was 13% survival in the vehicle treated group whereas in the peramivir treated group at 24, 48, and 72 h, the survival was 100, 40, and 50%, respectively. Survival in the oseltamivir groups at 24, 48 and 72 h was 90, 30 and 20%, respectively.

Table 1
Effect of peramivir treatment (IM) on weight loss and survival in mice infected with H1N1 virus (prophylaxis model).

Treatment	Dose level (mg/kg)	Percent survival	Mean weight change (±SEM) day 4
Vehicle, uninfected	0	100	0.32 ± 0.22
Vehicle, infected saline treated	0	20	−3.41 ± 0.17
Peramivir	1.0	60*	−2.11 ± 0.22**
Peramivir	3.0	60*	−1.51 ± 0.33**
Peramivir	10.0	90**	−1.84 ± 0.19**
Peramivir	30.0	100**	−1.83 ± 0.17**

* $p < 0.03$ vs. vehicle, infected control group.
** $p < 0.001$ vs. vehicle, infected control group.

Conclusion: These data demonstrate efficacy of parenterally administered peramivir against the recently isolated pandemic flu virus.

doi:10.1016/j.antiviral.2010.02.361

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In Vitro Dose Ranging Studies for Serine Protease Inhibitor, MK-4519, Against a Hepatitis C Virus (HCV) Replicon using the Bellocell System

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Background: Development of new anti-HCV agents has been limited due to the inability to effectively grow HCV in cell culture. This problem is obviated by use of a HCV replicon system. We have developed an in vitro system to examine the pharmacodynamically-linked parameters and monitor the exposure response of MK-4519 against HCV using a HCV replicon-bearing cell line.

Methods: The HCV replicon cell line, 2209–23, was obtained from Roche (Palo Alto, CA). The replicon contains a Renilla luciferase reporter gene that was used to monitor HCV replication kinetics. We inoculated 2209–23 cells into four BelloCell bottles at a concentration of 6×10^7 cells per bottle. One bottle served as a control and three bottles were treated with various concentrations of MK-4519, which was obtained from Merck. Medium, with or without drug, was infused into the system for 14 days. Replicon kinetics and cell growth were monitored daily by harvesting 6 carrier flakes in quadruplicate. Three sets of 6 flakes were trypsinized to remove 2209–23 cells from the flakes and viable cells were counted. RNA was later extracted from these trypsinized cells and used for sequencing of the NS3 gene. The remaining flakes were immersed in Renilla luciferase lysis buffer and frozen until the end of the study. Luciferase activity was quantified from all samples simultaneously to determine the effect of each drug on the HCV replicon.

Results: In the BelloCell system, MK-4519 significantly reduced luciferase activity in the 2209–23 cells in a dose dependent manner. Cell viability assays confirmed that the suppression of luciferase activity was not due to cytotoxicity. Sequencing analysis detected genotypic changes in the replicon as a result of drug exposure. Mutation A156T, which is associated with phenotypic drug resistance, was present only in replicons exposed to drug.

Conclusion: These studies indicate that the BelloCell system is an effective and relevant in vitro method to model in vivo pharmacodynamics for antiviral agents active against HCV. This system can be used to optimize dosing for anti-HCV compounds for inhibition of viral replication and suppression of resistance.

doi:10.1016/j.antiviral.2010.02.362

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Maporal Hantavirus ββ-Integrin Utilization and Sensitivity to Favipiravir

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Hantaviruses are members of the Bunyaviridae family of viruses. Pathogenic hantaviruses are the etiologic agents of hemorrhagic fever with renal syndrome (HFRS), a disease principally endemic in the Old World, and hantavirus pulmonary syndrome (HPS), a disease primarily restricted to the Americas. Maporal virus (MPRLV), a recently isolated hantavirus, has been found to cause disease in hamsters that resembles HPS in humans. However, the virus has not been linked to human cases of HPS. Considerable evidence suggests that ββ-integrin usage mediating infection may serve to distinguish pathogenic from non-pathogenic hantaviruses, but this receptor usage pattern information is not yet available for MPRLV. Although ribavirin has been shown to be effective in treating HFRS, it lacks specificity and has toxicity. Moreover, there are no effective antivirals for the treatment of HPS. Considering the above, we have investigated MPRLV (1) susceptibility to favipiriavir (T-705), (2) ββ-integrin-mediated mechanism of entry, and (3) genetic determinants of pathogenicity. Favipiravir, a pyrazine derivative reported to be active against related bunyaviruses, was found to be active against MPRLV, Dobrava virus (DOBV), and Prospect Hill virus (PHV) (EC_{50} = 65–93 μM) with therapeutic indexes of 77, 65, and 82, respectively. Using antibodies targeting specific integrin chains, we found infection of Vero E6 cells with MPRLV to be dependant on ββ3 integrins, similar to that reported for other pathogenic hantaviruses such as DOBV included in our studies. ββ1-Integrin chain-specific antibodies and fibronectin did not block MPRLV or DOBV infectivity as observed with the non-pathogenic PHV. Phylogenic analysis of characteristic degron sequences and ITAM motifs in the G1 cytoplasmic tails of MPRLV and other hantaviruses emphasizes the close genetic proximity of MPRLV to other HPS-causing hantaviruses. The data presented suggests that MPRLV may be pathogenic to humans and that it and other hantaviruses tested are sensitive to favipiravir in cell culture.

Acknowledgement: Supported in part by N01-AI-30048, Virology Branch, NIAID, NIH.

doi:10.1016/j.antiviral.2010.02.363

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Small Molecule Inhibitors of Dengue Virus Replication are Active *In Vivo*

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Flaviviruses, including dengue virus, West Nile virus, yellow fever virus, Japanese encephalitis virus, and tick-borne encephalitis virus, are a group of viruses transmitted by mosquitoes or ticks in the Flaviviridae family, that are significant pathogens responsible for emerging infectious disease in both humans and animals. Dengue virus is considered to be one of the most important viruses