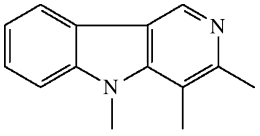
49

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

Masanori Baba[1,*], Mohammed Salim[1], Mika Okamoto[1], Hiroshi Aoyama[2], Yuichi Hashimoto[2], Yutaka Takebe[3], Jan Paeshuyse[4], Johan Neyts[4]

[1] *Kagoshima University, Kagoshima, Japan;* [2] *The University of Tokyo, Tokyo, Japan;* [3] *National Institute of Infectious Diseases, Tokyo, Japan;* [4] *Katholieke Universiteit Leuven, Leuven, Belgium*

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

50

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

Shanta Bantia*, Debra Kellogg, Cynthia Parker, YuHong Luo, Y.S. Babu

BioCryst Pharmaceuticals, Inc., Birmingham, USA

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

51

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

Shanta Bantia[1,*], Debra Kellogg[1], Cynthia Parker[1], Ramanda Upshaw[1], Natalia Ilyushina[2], Mark Prichard[3], Y.S. Babu[1]

[1] *BioCryst Pharmaceuticals, Inc., Birmingham, USA;* [2] *St. Jude's Children's Research Hospital, Memphis, USA;* [3] *University of Alabama at Birmingham, Birmingham, USA*

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.