97

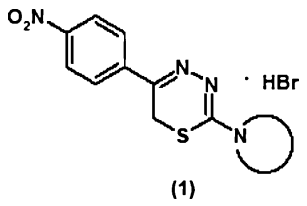
Anti-orthopoxviral activity of the 2-Cycloalkylimino-5-(4-Nitrophenyl)-1,3,4-Thiadiazine Derivatives

Evgeny Belanov [1,*], Lyudmila Sidorova [2], Vladimir Rusinov [2], Nikolay Bormotov [1], Sergey Balakhnin [1], Olga Serova [1], Valery Charushin [2], Oleg Chupakhin [2]

[1] SRC VB, Koltsovo, Novosibirsk reg., 630559, Russia; [2] Ural State Technical University, Yekaterinburg 620002, Russia

During this study, we synthesized a series of the 2-cycloalkylimino-5-(4-nitrophenyl)-1,3,4-thiadiazinesof the general formula (1) wherein the group (N) represents: piperidino-, pyrrolidino-, methylpiperazino-, hexamethyleneimino-group. These derivatives were tested for cytotoxicity and antiviral activity against the orthopoxviruses: vaccinia, cowpox and mousepox in cell cultures. Some of the derivatives show antiviral activity which depended from type of viruses and from the structural features of the compounds. Thus, we find a new class of heterocyclic compounds with antiviral activity against the orthopoxviruses.

Acknowledgment: This research was funded by ISTC Project #1989/BTEP#23.



(1)

doi:10.1016/j.antiviral.2008.01.111

98

Resistance of Human Cytomegalovirus to Cyclopropavir Involves a Novel Mutation in UL97

Katherine Z. Borysko [1,*], Brian G. Gentry [1], Julie M. Breitenbach [1], Jiri Zemlicka [2], John C. Drach [1]

[1] Department of Biologic & Materials Sciences, School of Dentistry, University of Michigan, Ann Arbor, USA; [2] Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, USA

We have previously described a second-generation methylenecyclopropane with two hydroxymethyl groups on the cyclopropane ring. This guanine nucleoside analog termed cyclopropavir (CPV) was active in vitro against HCMV and MCMV with IC_{50}'s of 0.27–0.49 μM and no cytotoxicity at 100 μM [*J. Med. Chem.* **47** (2004) 566]. It also was active when administered orally to MCMV-infected mice [*Antimicrob. Agents Chemother*. **48** (2004) 4745]. Last year we reported the isolation of HCMV resistant to CPV by passage of Towne strain HCMV in selected concentrations of the drug. Dose–response experiments with plaque-purified virus (termed 2696^r) gave IC_{50}'s of 22 and 42 μM for CPV and ganciclovir (GCV) compared to IC_{50}'s of 0.9 and 1.5 μM, respectively, for wt virus [*Antiviral Res.* **74** (2007) A83]. The virus also was resistant to two first generation analogs. Data such as these and the fact that both CPV and GCV are guanine nucleoside analogs led us to hypothesize that like GCV, CPV is phosphorylated to its active form by the CMV kinase pUL97. Consequently we sequenced UL97 from twice plaque-purified 2696^r and found a deletion of base pair 498. This deletion produced a frame shift resulting in the acquisition of a stop codon at base pairs 502-4 that normally occurs at base pairs 2122-4. The resulting putative protein would be 168 amino acids in length (normally 708) and would contain neither the ATP-binding region (codons 460–520) nor the substrate-recognition site (codons 590–607) found in wild-type UL97. Nonetheless virus 2696^r grew at nearly the same rate and to a titer only one $\log_{10}$ lower than wt virus. In contrast HCMV with UL97 deleted (kindly provided by Mark Prichard) grew much more slowly and to a titer several $\log_{10}$'s lower. These data demonstrate virus 2696^r is resistant to both GCV and CPV plus imply that it can replicate with a severely truncated pUL97.

Acknowledgements: Supported by grants P01-AI46390 and R44-AI054135 from NIH and funds from the University of Michigan.

doi:10.1016/j.antiviral.2008.01.112

99

Synthesis and Activity of Vidarabine D-Amino Acid Prodrugs as Potential Pox Virus Agents

Julie M. Breitenbach [1,*], Wei Shen [2], John Hilfinger [2], John C. Drach [1]

[1] Department Biologic & Materials Sciences, University of Michigan, Ann Arbor, USA; [2] TSRL, Inc., Ann Arbor, USA

Vidarabine (ara-A) was originally developed as an anti-tumor agent and later was found to be active against HSV types 1 and 2. It was the first FDA-approved drug for treatment of systemic herpes infections but its use is limited due to metabolism by adenosine deaminase (ADA) to ara-H, low lipophilicity, and low intestinal membrane permeability. Its low aqueous solubility also limits formulation options. We recently reported that vidarabine was three- to fivefold more active in plaque reduction assays against vaccinia and cowpox viruses than was cidofovir [Antiviral Res. 70 (2006) A14]. Furthermore, its activity against these viruses was enhanced approximately 10-fold by combination with 1 μM 2′-deoxycoformycin, a potent inhibitor of ADA. We also reported that minimizing the conversion of vidarabine to ara-H by synthesizing 5′-L-amino acid substituted prodrugs gave more potent anti-pox activity. We showed that the prodrugs are resistant to inactivation by deamination and that delivery of the L-amino acid prodrugs to the small intestine resulted in a 10-fold increase in the vidarabine plasma levels when compared with vidarabine. We now report the synthesis and antiviral activity of D-amino acid prodrugs of vidarabine as well as the 5′-valerate analog. The 5′-valerate and the D-valyl, -leucyl, -isoleucyl, phenylalanyl, -alpha and beta aspartyl prodrugs all were active against vaccinia and cowpox viruses at non-cytotoxic concentrations. Time-of-addition studies indicated the activity of vidarabine against vaccinia