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Rhinovirus 3C Protease Inhibitors Are Efficacious Against Several Related Picornaviruses.

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The *Picornaviridae* family consists of greater than 200 viral serotypes, which are associated with a wide variety of medically important diseases including the common cold, aseptic meningitis, conjunctivitis, encephalitis, and respiratory disease. The picornavirus 3C protease is responsible for the cleavage of viral precursor polyproteins into structural and enzymatic proteins which are essential for viral replication. DNA sequence comparisons among several related picornaviruses identified a significant degree of homology within the 3C coding region including the strict conservation of the cysteine, histamine, and glutamic acid residues which comprise the 3C catalytic triad. AG6084 was developed as a specific rhinovirus 3C protease inhibitor with potent activity against the enzyme (K_i = 6 nM) and with broad antiviral activity against multiple rhinovirus serotypes with ED_{50}s ranging from 2.1 to 6.3 μM. We have extended these studies and have evaluated the antiviral activity of AG6084 against the replication of several other picornaviruses including coxsackieviruses A21 and B3 in HeLa cells as well as enterovirus 70 and echovirus 11 in MRC-5 cells. AG6084 effectively inhibited the replication of all tested picornaviruses with ED_{50}s ranging from 1.1 to 5.0 μM. Virus specificity was demonstrated by the inability of AG6084 to inhibit the replication of an unrelated virus, herpes simplex virus, with an ED_{50} > 100 μM. SDS-PAGE analysis of radiolabeled proteins from cells infected with the different picornaviruses showed that AG6084 inhibited 3C-mediated proteolytic processing as evidenced by the accumulation of viral precursor polyproteins and the reduction of processed products. These results highlight the advantages of 3C protease as a target and also indicate that inhibitors designed toward rhinovirus 3C protease may be effective as potential therapeutics against a wide variety of other medically important picornaviruses.

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In Vitro Isolation And Characterization Of A Human Rhinovirus Variant Resistant To A 3C Protease Inhibitor.

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Human rhinoviruses (HRV) are the most important etiological agents of the common cold. Currently, no specific antivirals are available for the treatment of rhinovirus infection. The rhinovirus 3C protease is responsible for the cleavage of viral precursor polyproteins into structural and enzymatic proteins which are essential for viral replication. AG6084 has been demonstrated to be a specific inhibitor of 3C protease with potent activity against the enzyme (K_i = 6 nM) and broad antiviral activity against multiple rhinovirus serotypes (ED_{50}s = 2.1 to 6.3 μM). To select for an HRV strain with reduced sensitivity to AG6084, HeLa cells were infected with HRV-14 and serially passaged in the presence of increasing concentrations of drug. After 6 passages (p6), a virus variant was isolated which demonstrated a 3-fold reduction in sensitivity to AG6084. The p6 variant did not appear to be impaired for growth since levels of infectious virus produced following infection of HeLa cells with equal m.o.i.s of either p6 or wild-type viruses were similar. This finding was consistent with SDS-PAGE analysis of radiolabeled extracts from cells infected with either p6 or wild-type viruses which demonstrated no significant quantitative differences in viral protein synthesis. RT-PCR amplification of 3C protease from p6 and wild-type viruses followed by DNA sequence analysis of individual virus clones revealed threonine to alanine substitutions at positions 129, 131, and 143 and a lysine to arginine substitution at position 174. These amino acid changes are consistent with molecular modeling analyses which indicate that amino acids 129, 131 and 143 are located in the 3C active site and interact with AG6084 at P1 and P2. The relevance of the change at position 174, located away from the 3C active site, remains to be determined. Drug susceptibility assays on recombinant viruses constructed to contain the observed mutations are in progress.

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Studies on Antiviral Effect of Extract from *Alternanthera philoxeroides* Griseb on Epidemic Hemorrhagic Fever Virus *in Vivo*.

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Antiviral effect of extract of *Alternathera philoxeroides*(Mart.) Griseb(APG) was studied in lethal epidemic hemorrhagic fever virus(EHFV) infection model of suckling mice. Outbred Kunming suckling mice below 72 hours old were infected with 0.04ml (100 $ICLD_{50}$/0.02ml) $EHFV_{B\text{-}114}$ intraperitoneally(i.p.) for each animal. 2.5g/Kg/day APG extract was i.p. administrated at 6 hours, 2 days, 6 days, 10 days and 14 days after infection respectively. Herbal extract administration lasted for 8 days. When the herbal extract was given within 10 days postinfection, 51.72%-79.41% of infected mice survived longer with the mean survival times of 30.7±7.2-37.4±4.9 days. While none of the untreated control group survived with the mean survival times of only 21.2±1.7 days($p < 0.01$). However, when the herbal extract was administrated 14 days postinfection, there were no significant differences between this group and the control group ($p > 0.05$). The APG extract administration could interrupt viremia and eliminated the infectious virus in the tissues, mitigated pathological lesion and virus antigen in the tissues as compared with the control group. Additionally, the earlier the herbal extract was administrated, the more significant the antiviral effects were. These results suggest that APG extract is effective in the treatment of EHFV infection in suckling mice.

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Inhibition of Pestivirus (BVDV) Replication by a Cyclic Urea Derivative

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A cyclic urea derivative (CU) is a potent inhibitor of replication of bovine viral diarrhoea virus (BVDV) in calf trachea cell line (CT cells). IC90 value <0.032 μg/ml was recorded in the one step growth cycle experimental setup. A very high selectivity ratio exceeding 1560, was found. This activity correlate with the marked protective effect of this compound administered orally both in experimental BVDV infection in calves and in field trials on calves with natural mucosal diseases (viral diarrhoea).

The drug-sensitive period in BVDV replication cycle embraced the latent period and the exponential phase. Moreover, a 90-minute delay in the peak of viral RNA synthesis was observed in the CU treated cells.