

論 文 要 旨

Studies on novel antiviral agents against bovine viral diarrhea virus (BVDV) and hepatitis C virus (HCV)

新規抗ウシウイルス性下痢症ウイルス (BVDV) 薬
および抗 C 型肝炎ウイルス (HCV) 薬に関する研究

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Introduction and Objectives

Hepatitis C virus (HCV) infection represents a major public health problem. Nearly 3% of the population is chronically infected with this virus worldwide. The HCV vaccine is not available so far, and current available treatments are unsatisfactory. These facts mandate the urgent need for novel anti-HCV agents. Due to several characteristics shared between bovine viral diarrhea virus (BVDV) and HCV with respect to virion structure, genome organization, and replication cycle, BVDV is considered as a good surrogate model for HCV and has been used for investigating anti-HCV agents. In addition, the establishment of subgenomic and full-genomic HCV replicon cells as well as the infectious strain JFH1 is also accelerates the discovery of novel anti-HCV agents. The objectives of our study are 1) to find selective inhibitors of BVDV and/or HCV, 2) to examine a variety of compounds for their inhibitory effect on HCV replication in replicon cells, and 3) to elucidate the mechanism of action of the active compounds.

Materials and Methods

1. Anti-BVDV study

Anti-BVDV activity and cytotoxicity of test compounds were examined in MDBK cells by LDH and MTT colorimetric assays, respectively. The activity was confirmed by real-time reverse transcription (RT)-PCR and virus yield reduction assay. The time of drug-addition experiment and docking study for active compounds were also performed.

2. Anti-HCV study

Anti-HCV activity and cytotoxicity of test compounds were examined in subgenomic replicon cells by luciferase-based reporter assay and MTT assays, respectively. The activity was confirmed in different subgenomic and full-genomic replicon cells by real-time RT-PCR. The active compounds were also examined for their inhibitory effects on NS3 protease and NS5B RNA polymerase activity by biochemical assays.

Results

Some of the γ -carboline derivatives were found to be potent and selective inhibitors of BVDV replication. Among the compounds, 3,4,5-trimethyl- γ -carboline was found to be the most active against BVDV (Nose strain) in MDBK cells, with a 50% effective concentration (EC_{50}) of $0.017 \pm 0.005 \mu\text{M}$ and 50% cytotoxic concentration (CC_{50}) of $7.4 \pm 0.9 \mu\text{M}$. The compound inhibited viral RNA synthesis in a dose-dependent fashion. A time of drug-addition experiment and antiviral assay of the γ -carboline derivatives against the mutant strains resistant to some classes of BVDV RNA-dependent RNA polymerase inhibitors suggest that the compounds may target the RNA-dependent RNA polymerase.

A number of compounds were examined for their inhibitory effect on HCV replication in Huh-7 cells harboring subgenomic viral RNA replicons with a luciferase reporter (LucNeo#2). Among the compounds, some phenanthridinone derivatives were found to be active. The EC_{50} of the most active derivative was $0.063 \pm 0.010 \mu\text{M}$. This compound did not show apparent cytotoxicity to the host cells at concentrations up to $40 \mu\text{M}$. Its potent and selective anti-HCV activity was confirmed by real-time RT-PCR in different replicon cells. Interestingly, the phenanthridinone derivatives were less potent inhibitors of genotype 2a than genotype 1b of HCV. Since the phenanthridinone derivatives did not inhibit NS3 protease or NS5B RNA polymerase activity in biochemical assays, their molecular target (mechanism of inhibition) remains unknown

Discussion and Conclusion

In initial studies, we used BVDV as a surrogate model to HCV and tested compounds for their anti-BVDV activity. Although some compounds including the γ -carboline derivatives were potent and selective inhibitors of BVDV, they could not inhibit HCV replication. Therefore, we also examined directly the anti-HCV activity of compounds in HCV replicon cells. After screening a number of compounds, we could identify novel phenanthridinone derivatives as highly potent and selective inhibitors of HCV. The compounds were unique in their mechanism of action, since they did not inhibit HCV polymerase or protease, both of which are major targets for inhibition of HCV replication by antiviral agents currently under development.

In conclusion, compounds active against BVDV are not always good inhibitors of HCV due to the genomic variability between the two viruses. However, these compounds may be useful in the field of veterinary medicine and/or should be subjected to chemical modification to confer anti-HCV activity.

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