Direct-acting Antivirals and Host-targeting Agents against the Hepatitis A Virus

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Abstract

Hepatitis A virus (HAV) infection is a major cause of acute hepatitis and occasionally leads to acute liver failure in both developing and developed countries. Although effective vaccines for HAV are available, the development of new antivirals against HAV may be important for the control of HAV infection in developed countries where no universal vaccination program against HAV exists, such as Japan. There are two forms of antiviral agents against HAV: direct-acting antivirals (DAAs) and host-targeting agents (HTAs). Studies using small interfering ribonucleic acid (siRNA) have suggested that the HAV internal ribosomal entry site (IRES) is an attractive target for the control of HAV replication and infection. Among the HTAs, amantadine and interferon-lambda 1 (IL-29) inhibit HAV IRES-mediated translation and HAV replication. Janus kinase (JAK) inhibitors inhibit La protein expression, HAV IRES activity, and HAV replication. Based on this review, both DAAs and HTAs may be needed to control effectively HAV infection, and their use should continue to be explored.

Keywords: Amantadine; DAA; HAV; HTA; La protein; Drug overview.

Introduction

Hepatitis A virus (HAV) infections remain a major cause of acute hepatitis and occasionally lead to acute liver failure (ALF).1 Liver transplants are occasionally used in the treatment of HAV-associated ALF.2 Acute kidney injury,3,4 hemophagocytic syndrome,5 and pure red cell aplasia6 have been associated with HAV infection. HAV is usually transmitted by the fecal-oral route, and it was found that the prevalence of HAV infection decreased with the improvement of sanitation conditions in developed countries.7 Despite the successful results of childhood hepatitis A vaccination programs in the United States, the mean age at death among decedents with HAV infection increased to 76.2 years in 2011.7 HAV-related mortality declined, however, suggesting that older patients are more susceptible to HAV infection and more severely affected.7,8

In Japan, no universal vaccination program against HAV exists, and only 14% of indigenous Japanese people have received anti-HAV vaccinations.9 The positive rate of anti-HAV in patients under 30 years (0-7%) was found to be lower than those over 50 years (33%) in the indigenous Japanese population.9 Therefore, the possibility of an outbreak of an HAV epidemic exists.10,11 A similar situation exists in South Korea.12 Although there are safe and effective vaccines against HAV, it is also important to discover new host cell targets and to develop potential drugs for the treatment of HAV.13-15

Hepatitis A virus (HAV) infection is a member of the Hepatovirus genus of the Picornaviridae family. There are at least six genotypes of HAV, and three of them (I to III) are of human origin.16,17 HAV is a positive single-stranded, nonenveloped ribonucleic acid (RNA) virus of ~7,500 bases in length. The HAV genome codes one open reading frame that encodes structural (viral protein (VP)4, VP2, VP3, and VP1) and nonstructural proteins (2A, 2B, 2C, 3A, 3B, 3C, and 3D) and is flanked by a 5′ untranslated region (UTR) and a 3′ UTR. The HAV genome is translated into a single polyprotein in a cap-independent manner, i.e. HAV exhibits IRES-mediated translation. Subsequently, the single HAV polyprotein is proteolytically processed by protease 3C and cellular protease(s) into several functional and mature proteins.13,18,19 HAV IRES-mediated translation and HAV RNA replication are important for HAV virion formation (Fig. 1). HAV 3D is the RNA-dependent RNA polymerase.18,19 In fact, HAV IRES and HAV 3C are attractive targets of antiviral drugs against HAV.

Antivirals against HAV (Table 1, Fig. 2)

Two forms of antiviral agents against HAV exist: direct-acting antivirals (DAAs) and host-targeting agents (HTAs). DAAs...
specifically target HAV and include protease inhibitors, a polymerase inhibitor, and IRES inhibitors. DAAs have none of the adverse events associated with interferon, such as flu-like syndrome, hematologic effects, or depression. However, studies of human immunodeficiency virus (HIV) and hepatitis C virus (HCV) suggest that several DAAs exhibit genotype-specific antiviral activities with low genetic barriers to resistance.\(^\text{20-22}\) HTAs have high genetic barriers to resistance and exhibit pan-genotypic antiviral activities. HTAs have mechanisms of action that are complementary to those of DAAs, and HTAs typically act in a synergistic manner with DAAs.\(^\text{23}\) In order to effectively control HAV, it is important to develop both DAAs and HTAs.

**DAAs against HAV**

**HAV 3C protease inhibitors**

HAV 3C proteinases play an important role in the processing of the HAV polyprotein. Inhibitors of HAV 3C can result in the suppression of HAV replication, and there are several reports available regarding inhibition of HAV 3C.\(^\text{24-34}\) The binding of the peptide aldehyde Ac-Leu-Ala-Ala-(N,N-dimethyl-glutaminal) to the HAV 3C proteinase leads to...

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**Table 1. Effective antiviral agents against hepatitis A virus (HAV)**

<table>
<thead>
<tr>
<th>Direct-acting antivirals (DAAs)</th>
<th>Host-targeting agents (HTAs)</th>
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<tbody>
<tr>
<td>HAV 3C cysteine protease inhibitors(^\text{24-33})</td>
<td><strong>Broad-target HTAs</strong></td>
</tr>
<tr>
<td>-</td>
<td>Interferon-alpha(^\text{45,46})</td>
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<tr>
<td>Small interfering RNAs against HAV(^\text{48,42})</td>
<td>Interferon-gamma(^\text{52})</td>
</tr>
<tr>
<td>Targets: 2C, 3C and IRES</td>
<td>Interferon-lambda 1 (IL-29)(^\text{53})</td>
</tr>
<tr>
<td>-</td>
<td>Ribavirin(^\text{58-60})</td>
</tr>
<tr>
<td>-</td>
<td>Amantadine(^\text{58-64})</td>
</tr>
<tr>
<td>-</td>
<td><strong>More precisely targeted HTAs</strong></td>
</tr>
<tr>
<td>-</td>
<td>Agents against key host enzymes(^\text{14})</td>
</tr>
<tr>
<td>-</td>
<td>Agents against key cellular factors</td>
</tr>
<tr>
<td>-</td>
<td>Target: La(^\text{15,65})</td>
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\(^*\) Suppression of HAV internal ribosomal entry-site (IRES).
reversible and slow-binding inhibition of HAV 3C. A peptidyl monofluoromethyl ketone (peptidyl-FMK) inhibitor analogous to the peptide aldehyde has the ability to suppress HAV polyprotein processing and HAV replication. HAV replication is reduced 25-fold in the presence of 5 µM peptidyl-FMK in subclone 11-1 fetal rhesus monkey kidney cells (FRhK-4-cells) at day 1 postinfection. Beta-lactones also represent a new class of cysteine proteinase inhibitors that act on HAV 3C cysteine proteinases. Blaum et al. identified the hexanucleotide 5'-GGGGGT-3' (G(5)T) as an HAV 3C protease inhibitor and reported that the sequence-specific small nucleic acid-protein interaction mediated by this hexanucleotide may suppress HAV replication. Thus, an HAV 3C protease inhibitor is an attractive DAA.

**HAV-specific small interfering RNAs (siRNAs)**

In general, siRNAs can specifically knockdown target genes and have significantly affected biological and pharmacological research. Gene knockdown is achieved using 21 nucleotide double-stranded RNA (dsRNA) intermediates that are known as siRNAs, and they do not activate the interferon signaling pathway. Such siRNAs prevent a target gene from producing its functional protein. RNA interference (RNAi) may effectively treat viral infection with or without traditional antiviral therapies, although delivery of siRNAs to target cells is difficult.

Initially, we made and examined the effects of several siRNAs that targeted HAV nonstructural protein-coding regions related to HAV replication. Our studies revealed that siRNAs against the HAV 2C- and 3D-coding regions inhibited HAV 2C and HAV 3D expression and that the combination of 2C-siRNAs and 3D-siRNAs strongly inhibited HAV replication. Although consecutive siRNA applications select mutants that either preexist as quasispecies of the HAV genome or are generated during genome replication in HAV infection, consecutive siRNA transfections targeting multiple sequences in the HAV genome may result in a more efficient and sustained silencing effect than a single transfection.

**HAV-specific siRNAs suppress HAV IRES-mediated translation and HAV replication**

We showed that various siRNAs that target the HAV IRES suppressed HAV IRES-mediated translation and HAV replication. RNase III (endoribonuclease)-prepared siRNAs (esiRNAs) that are targeted to various domains of the HAV IRES efficiently suppressed replication-competent HAV replicon replication to 42% (0.5 µg/mL esiRNA) and 12% (1.0 µg/mL esiRNA) of the control level at 48 h post-infection.

In a previous study, we made several vector-derived short hairpin RNAs (shRNAs) that targeted HAV IRES and examined their effects on HAV replication. Although several shRNAs that targeted the loop regions of stem-loop structures of the HAV IRES were made, the shRNAs that targeted the HAV IRES domains IIIc and V were found to efficiently suppress genome translation and replication. This study suggested that the HAV IRES domains might serve as attractive targets for suppression of HAV replication and HAV infection.

**HTAs against HAV**

**Type I interferon**

The eradication of HAV from human hepatocytes is associated with interferon systems. It has been reported that interferon-alpha exhibits antiviral activity against HAV replication in the human hepatoma cell line PLC/PRF/5. Interferon-alpha suppresses HAV replicon replication and HAV replication, although interferon-alpha seems to have no additive effect on the suppression of HAV IRES-mediated translation by amantadine. Interferon was found to be clinically effective for the suppression of HAV infection in some cases, but it is contraindicated for severe HAV infections, including fulminant hepatitis, due to its adverse effects or its impairment of the interferon systems.

**Other types of interferons**

Interferon-gamma is produced upon HAV stimulation by HAV-specific human leukocyte antigen (HLA)-dependent T8+ (cytotoxic) T-lymphocytes and plays a role in the eradication of HAV infection. Recombinant interferon-gamma exhibits antiviral effects against persistent HAV infection in human fibroblasts.

It was recently reported that interferons-lambda [i.e., interferon-lambda 1 (IL-29), interferon-lambda 2 (IL-28A), and interferon-lambda 3 (IL-28B)] can inhibit HCV IRES-mediated translation and that IL-29 and IL-28A but not IL-28B can inhibit HAV IRES-mediated translation. In the same study, 100 ng/mL of IL-29 led to a 23% inhibition of HAV subgenomic replication, and 250 ng/mL and 500 ng/mL of IL-29 tended to inhibit HAV propagation without cell
damage. Because interferons-lambda use a receptor complex that is different from that of interferon-alpha and the majority of bone marrow-derived cells and nerve cells do not express the receptors for interferons-lambda, interferon-lambda resulted in fewer adverse events, such as the hematological cytotoxicities or depression, compared with interferon-alpha. Therefore, interferons-lambda may be useful for severe conditions resulting from HAV infection. Further studies are needed.

Ribavirin

Ribavirin (1-beta-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) is a broad-spectrum synthetic guanosine analog that acts against deoxyribonucleic acid (DNA) and RNA viruses. At 100 µM, ribavirin moderately inhibited HAV propagation. The effects of ribavirin on HAV replication may be non-specific, and ribavirin has no effect on HAV IRES-mediated translation.

Amantadine

Amantadine is a tricyclic symmetric amine and is also a broad-spectrum antiviral that acts against influenza A viruses. Several groups have reported the effects of amantadine on the growth of HAV in cell culture models. Amantadine inhibited viral antigen synthesis when added to cells after the attachment step, suggesting that amantadine acts during or after the HAV entry pathway. Amantadine also inhibited HAV IRES-mediated translation and HAV replication. Amantadine can inhibit clinical isolate-derived HAV IRES-mediated translation, and although the effects of amantadine may be strain-dependent, the influence of HAV genotypes and sequence variations on the effects of amantadine should be explored. The combinations of amantadine with interferon-alpha or IL-29 resulted in stronger inhibitory effects on HAV replication compared with amantadine alone. Broad-target HTAs, such as interferon, ribavirin and amantadine, may suppress HAV replication in certain HAV patients.

Agents against key host enzymes and cellular factors

The key host enzymes and cellular factors that are required for the viral lifecycle are targets of antiviral therapies. Several cellular proteins, such as autoantigen La, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), poly(pyrimidine tract-binding protein (PTB)/hnRNPI), poly(C) binding protein 2 (PCBP2/hnRN-E2), polyadenylate-binding protein-1 (PABP), eukaryotic translation initiation factor 4E (eIF4E), and eukaryotic translation initiation factor 4G (eIF4G), interact with HAV IRES RNA. These proteins might be associated with HAV replication. SiRNAs against La strongly inhibited HAV IRES activities and HAV subgenomic replication. The janus kinase (JAK) inhibitors SD-1029 and AG490 reduced La protein expression and inhibited HAV IRES activities and HAV replication in African green monkey kidney GL37 cell lines. We also found that the JAK2 inhibitor AZD1480 inhibited the expression of phosphorylated-(Tyr-705)-signal transducer and activator of transcription 3 (STAT3) and La and inhibited HAV IRES-mediated translation and HAV replication in human hepatoma cell lines. Thus, La plays a role in HAV replication and might be an important target of HAV therapy.

Ammonium chloride, methylamine, and dansylcadaverine also inhibit HAV protein synthesis after the attachment step. The effects of phospholipase A2, phospholipase C, trypsin, and beta-galactosidase on HAV infection have also been observed, and suggest that these drugs act on HAV attachment on the cellular surface. Monensin acts as an ionophore on intracellular vesicle compartments and may inhibit HAV infection at the uncoating step. Isoflavans and isoflavenes have inhibitory effects on the penetration and/or uncoating step of HAV infection. Cell membrane lipid components might also be attractive targets due to their interactions of HAV. It has previously been reported that glycyrrhizin, pyrazofurin, arabinosylcytosine, and carrageenan exhibit antiviral activities against HAV.

HAV and HCV replication were similarly sensitive to interferons, but clear differences existed for dependency on phosphatidylinositol 4-kinase IIα and β (PI4KIII), miR122, and immunophils. HAV replication was inhibited by the "oral formulation" silibinin, a flavonolignan isolated from the milk thistle, *Silybum marianum*. Thus, there might be many reagents that interact with HAV infection. Further studies are needed.

Conclusions

There are six HAV genotypes (I-VI): HAV genotypes I-III could infect humans, although only one serotype exists in HAV. Further molecular epidemiological investigations and evolutionary studies may provide a valuable opportunity to study diversified drug responses to different HAV genotypes. In this review, we selected the references about antiviral agents against HAV from PubMed online. DAAs and HTAs might be needed to control HAV infection. Although effective vaccines for HAV have been developed, antiviral agents against HAV should be explored until global eradication of HAV.

Conflict of interest

None

Author contributions

Drafting of the manuscript (TK), discussion and approval of the manuscript (TK, SN, SW, MN, XJ, YH, RS, OY).

References

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