Prenylation inhibitors: a novel class of antiviral agents

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Prenylation is a site-specific lipid modification of proteins. Although first described for a variety of cellular proteins, it has become apparent that viruses can also make use of this post-translational modification provided by their host cells. Depriving a virus access to prenylation can have dramatic effects on the targeted virus’s life cycle. Selective pharmacological inhibitors of prenylating enzymes have been developed and shown to have potent antiviral effects in both in vitro and in vivo systems. Because prenylation inhibitors target a host cell function, are available in oral form and are surprisingly well tolerated in human trials, these compounds represent an attractive new class of antiviral agents with potential for broad-spectrum activity. After a brief outline of host cell prenylation pathways, we review below the development of prenylation inhibition as an antiviral strategy applied to a prototype target, hepatitis delta virus (HDV), and discuss the potential application of prenylation inhibitors to a broad range of other viruses.

Keywords: farnesyltransferase inhibitors, hepatitis delta virus, antiviral therapy

Prenylation as a post-translational modification of proteins

Prenylation is a post-translational lipid modification involving covalent addition of either farnesyl (15 carbon) or geranylgeranyl (20 carbon) prenyl lipids derived from mevalonic acid to conserved cysteine residues at or near the C-terminus of proteins. These reactions are catalysed by protein farnesyltransferase (FTase) and protein geranylgeranyltransferases (GGTases), respectively (Figure 1). The substrate for FTase or GGTase I is a characteristic tetrapeptide, the so-called CXXX box motif (where C is a cysteine and X is one of the last three amino acids at the C-terminus of the protein). A second class of GGTases, GGTase type II, has a more complex substrate recognition and catalyses the transfer of geranylgeranyl to cysteine residues contained in C-terminal motifs such as CC or CXC. Examples of farnesylated proteins include lamin B, yeast mating pheromone α-factor and Ras, while the γ-subunit of G proteins and the Rab proteins are examples of geranylgeranylated proteins. The effect of prenylation is to promote membrane association of the modified protein. Prenylation also plays a major role in protein–protein interactions.

Prenylation in HDV

Hepatitis delta virus (HDV) is an important cause of acute and chronic liver disease in various parts of the world and can dramatically worsen the liver disease associated with hepatitis B virus (HBV). It is estimated that ~15 million of HBV-infected individuals are infected with HDV worldwide, the majority of whom reside in the Mediterranean and Amazon basins, the Middle East, Central and West Africa, and Central Asia. In the USA, the prevalence of HDV has been estimated at 70,000. There is no effective medical treatment for acute or chronic HDV infection. As detailed below, the study of HDV’s molecular virology has revealed that prenylation plays a key role in the viral life cycle and offers an attractive opportunity for anti-HDV therapy.

HDV has a single-stranded circular RNA genome. The latter encodes two proteins, the small and large hepatitis delta antigens, which are identical except for the presence of an additional 19 amino acids at the C-terminus of the larger isoform. The complete virus particle is composed of a complex of genome and both delta antigen isoforms, which is encapsulated by a lipid envelope embedded with HBV surface antigen (HBsAg) envelope proteins. As the latter are not encoded by HDV, but rather by a co-infecting HBV, this provides a molecular explanation for why HDV infection occurs only in the presence of a co-existing HBV infection. Although they share most of their amino acid sequence in common, the two delta antigen isoforms have remarkably different functions. For example, while the small delta antigen is essential for genome replication, the large delta antigen (LHDAg) can trans-dominantly inhibit this process and can transactivate a variety of genes. With respect to HDV particle assembly, only the LHDAg can mediate assembly and release of HBsAg-enveloped particles. The critical molecular determinants for HDV particle assembly were first determined using the simplest model of HDV assembly, virus-like particles (VLPs). The latter consist of LHDAg and small surface antigen (the smallest of the three HBV envelope proteins), which together are the minimal elements required for particle assembly.

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Within the 19 C-terminal amino acids unique to the larger delta antigen isoform, a CXXX box motif has been identified. This motif, comprising the last four amino acids of LHDAg, was noted to be absolutely conserved across HDV isolates, suggesting that it reflected an important function in vivo. It was hypothesized that this conserved CXXX box was a substrate for prenylation and that such a lipid modification could help mediate interaction with the membrane-associated HBsAg required for HDV morphogenesis. Labelling studies with [3H]mevalonate—the metabolic precursor of prenyl lipids—showed that LHDAg was indeed subject to prenylation both in in vitro translation reactions and in intact cells. Site-directed mutagenesis demonstrated that genetic disruption of the CXXX box—such as by substitution of the cysteine and X is one of the last three amino acids at the C-terminus of the protein to be modified. These reactions are catalysed by protein-farnesyltransferase (FTase) and protein-geranylgeranyltransferase (GGTase), type I or II. The recognition sequence of FTase and GGTase I consists of the so-called ‘CXXX box’ where C is a cysteine and X is one of the last three amino acids at the C-terminus of the protein to be modified. Selected enzymes in the above pathways can be targeted by pharmacological inhibitors. The latter include statins, FTase inhibitors (FTIs) and GGTase inhibitors (GGTIs), which target HMGCoA reductase (a), farnesyltransferase (b) and geranylgeranyltransferases (c), respectively. FTI-mediated inhibition of farnesyltransferase prevents prenylation of LHDAg, which is essential for production of HDV virus particles. This latter approach has proven highly effective in in vitro and in vivo models of HDV, and human trials are currently being planned. See text for additional details.

Figure 1. Outline of major protein prenylation pathways and potential targets for antiviral therapy. (a) Prenyl lipid synthesis begins with mevalonate, which is derived from acetyl CoA and the action of HMGCoA reductase. Mevalonate synthesis represents the committed step in cholesterol and prenyl lipid production. Mevalonate is converted into a 5-carbon isopentenyl pyrophosphate subunit. (b) Condensation of three of the latter yields the prenyl lipid farnesyl pyrophosphate (Farnesyl-PP). (c) Addition of a fourth subunit yields geranylgeranyl pyrophosphate. Protein prenylation occurs by the covalent, post-translational addition of either farnesyl or geranylgeranyl to conserved cysteine residues at or near the C-terminus of proteins. These reactions are catalysed by protein-farnesyltransferase (FTase) and protein-geranylgeranyltransferase (GGTase), type I or II. The recognition sequence of FTase and GGTase I consists of the so-called ‘CXXX box’ where C is a cysteine and X is one of the last three amino acids at the C-terminus of the protein to be modified. The recognition sequence of FTase and GGTase I consists of the so-called ‘CXXX box’ where C is a cysteine and X is one of the last three amino acids at the C-terminus of the protein to be modified. Selected enzymes in the above pathways can be targeted by pharmacological inhibitors. The latter include statins, FTase inhibitors (FTIs) and GGTase inhibitors (GGTIs), which target HMGCoA reductase (a), farnesyltransferase (b) and geranylgeranyltransferases (c), respectively. FTI-mediated inhibition of farnesyltransferase prevents prenylation of LHDAg, which is essential for production of HDV virus particles. This latter approach has proven highly effective in in vitro and in vivo models of HDV, and human trials are currently being planned. See text for additional details.

Prenylation inhibition-based inhibition of HDV assembly

The need for an effective treatment for HDV has prompted an evaluation of the potential of prenylation inhibition-based antiviral therapy. As a first test of this hypothesis, a cell line that produces HDV VLPs was created. Next, a candidate inhibitor was identified by relying on the fact that prenylation of HDV consists of the addition of the prenyl lipid farnesyl to large delta antigen, a reaction catalysed by farnesyltransferase. BZA-5B, a farnesyltransferase inhibitor (FTI) originally developed to prevent prenylation of the farnesylated oncogene Ras, was chosen for this purpose. BZA-5B was shown to be a potent inhibitor of large delta antigen prenylation and able to specifically inhibit the prenylation-dependent production of HDV VLPs in a dose-dependent manner. By 50 µM BZA-5B, no particles could be detected. Controls for non-specific inhibition of protein synthesis and secretion or cell metabolism were not exhibited. The significant
difference between the molecular structures of BZA-5B and FTI-277 suggested that HDV assembly was indeed inhibited by their common FTI activity rather than some other feature of the inhibiting drugs. Thus in spite of the added complexity and assembly determinants of infectious HDV virions compared with VLPs, the former are also sensitive to pharmacological prenylation inhibition. Moreover, production of HDV genotype III virions that are associated with particularly severe clinical disease was as sensitive to prenylation inhibition as was that of HDV genotype I virions.17

Most recently, the antiviral efficacy of FTIs was evaluated in vivo in a new mouse model of HDV. This model was established by hydrodynamically transfecting HBV-transgenic FVB mice with HDV-encoding plasmids, leading to robust intrahepatic viral replication and viraemia.20 Cohorts of mice in which HDV viraemia had been established were then treated with single daily doses of the prenylase inhibitors FTI-277 or FTI-2153, or vehicle controls. Both agents were highly effective at clearing HDV viraemia. As expected, HDV inhibition exhibited duration of treatment dependence. In addition, similar alanine aminotransferase levels among treatment groups argued against a non-specific hepatotoxic effect of the FTIs as a potential cause for the clearance of viraemia.20 This is a dramatic and clear first in vivo confirmation of the potential of this novel class of antiviral agents. These results have obvious clinical relevance and importance for human HDV infections. Since, in the mouse model, newly produced virions cannot infect new hepatocytes, the effect of FTIs on viral-related liver injury cannot be evaluated fully. In human HDV infections, however, it is expected that inhibition of the critical steps of assembly and release in the virus life cycle would have a major impact on the course of HDV infection and its associated liver disease.

At least for chronic HDV infection, because of the dependence on HBV for providing a source of HBsAg, theoretically, effective eradication of HBV in an individual would be expected to eventually lead to clearance of HDV as well. Unfortunately, the currently widely used oral anti-HBV agent lamivudine, while effectively decreasing HBV DNA levels, leaves HBsAg largely unaffected,21 as such it would be predicted to have little effect on HDV. Indeed, when used alone or in combination with high-dose interferon, lamivudine does not improve disease activity or lower HDV-RNA levels in patients with chronic delta hepatitis.22,23

Because of the lack of an effective anti-HDV therapy and the promising preclinical data in mice, FTIs are currently being considered for trials in human patients. For such purposes, well-tolerated, orally available FTIs would be preferred. Fortunately, the requisite candidate drugs have already been developed and used in humans, albeit for a different purpose. The requirement for prenylation in transformation by oncogenic Ras has made the enzymes responsible for this modification important targets for antitumour drug design. A variety of prenylation inhibitors have been synthesized and been evaluated for clinical use in several Phase I/II and III trials.24 These studies provide important information when considering this class of drugs as antiviral agents. First, most studies have used oral regimens, emphasizing a convenient route of administration. Secondly, the use of FTIs was found to generally be quite safe, with collectively the main reported side effects being reversible, dose-dependent myelo-suppression, fatigue, reversible neurocortical toxicity, prolongation of QT and mild gastrointestinal toxicity. Moreover, side effects differed depending on the particular FTI used, suggesting the side effects might be more compound-related rather than a direct effect of inhibiting farnesylation.

Attractive features of FTIs and application to other viruses

Prenylation inhibition-based antiviral therapy varies from more classical approaches to antiviral treatment, as it seeks to deprive the virus access to a host function. By targeting a host cell enzyme rather than a virus-specific target, such a strategy may actually impose some interesting challenges for a virus attempting to develop resistance. This is because the targeted locus is not under control of the virus. Moreover, because the farnesyl moiety of LHDAG may serve more of a specific ligand role rather than simply as a mediator of transient interactions with membranes, simple substitution of geranylgeranyl under conditions of FTI-mediated inhibition of farnesylation may be insufficient for mediating HDV assembly. Certainly to date, no such mechanism of resistance has been observed.

The described strategy could affect host cell prenylation as well, theoretically causing intolerable side effects. Surprisingly, this does not appear to be the case, as FTIs are remarkably well tolerated by host cells in vitro,25 and more importantly, treated cancer patients in vivo.26 Perhaps this reflects the fact that most prenylated proteins in cells are modified by geranylgeranyl rather than farnesyl27 or that the existence of a family of prenyltransferase enzymes offers the possibility of ‘cross prenylation’ when a particular prenyltransferase is targeted by drug treatment.

Prenylation inhibition-based antiviral therapy has implications for other viruses besides HDV, which are found to have similarly prenylated proteins. Analysis of sequence databases reveals that a CXXX box motif is present in proteins of numerous other medically important viruses, as well as in agents with a potential for bioterrorism. For example, UL32, a gene product of herpes simplex virus (HSV), thought to be involved in virus particle formation, contains such a CXXX box.28 Interestingly, Farassati et al.29 have treated a Ras-transformed cell line infected with HSV-1 with FTI-1 in order to evaluate whether Ras is involved in HSV-1 infection. A significant decrease in HSV-1 titres in treated cells as well as decreased viral protein synthesis were demonstrated, leading to their conclusion that HSV-1 exploits the host cell Ras signalling pathway for infection.29 Although not specifically evaluated, treatment with FTI-1 may also have had more of a direct antiviral effect of inhibiting viral protein prenylation, contributing to the observed viral inhibition. This might suggest that in HSV-1, and possibly in other viruses, prenylation inhibitors could have a dual mechanism: inhibiting prenylation of a viral protein as well as the cellular pathways exploited by the virus.

Prenylation is also predicted to be involved in viral processes beyond assembly. For example, in hepatitis A virus and the animal enterovirus causing foot and mouth disease, it is the polymerase protein that bears a CXXX box. Because these viruses replicate their RNA in intimate association with host cell membranes, prenylation may help mediate membrane-associated RNA replication. Not all CXXX boxes undergo prenylation.1 Thus, formally, the identification of a CXXX box in the protein of a contemplated viral target should be complemented by direct demonstration that the protein is indeed subject to prenylation. Finally, motifs that mediate other types of prenylation in host cell proteins such as terminal CC and CXC are expected to be operational in viral counterparts as well.
Leading article

Other targets for antiviral therapy in the prenylation pathway

As the synthesis of mevalonate by the enzyme hydroxy methylglutaryl-CoA (HMGC-CoA) reductase is a committed step in both cholesterol and prenyl lipid biosynthesis pathways, it has been suggested that the use of HMGC-CoA reductase inhibitors, such as the statins, could be used to inhibit prenylation. Indeed, prenylation can be inhibited in vitro by lovastatin; however, cytotoxic doses are required. Because cholesterol needs can be met by exogenous supply via the low-density lipoprotein receptor system, synergy between statins and FTIs can be contemplated. Potential synergetic effects of FTIs might also be achieved with other types of drugs, which inhibit additional steps in the pathway of prenyl protein synthesis. For example, most prenylated eukaryotic proteins are further processed by proteolysis and methylation, which enhance the efficiency of their association with membranes. To the extent prenylated LHDAg and other viral proteins are indeed further processed by these mechanisms, compounds that inhibit these reactions may also prove useful as antiviral agents alone or in combination with FTIs.

To summarize, prenylation inhibitors target a host cell function, are available in oral form and are surprisingly well tolerated in human trials to date. Although first shown to be effective against HDV, a large number of other viruses appear to contain proteins that are subject to prenylation. Thus, prenylation inhibitors represent an attractive new class of antiviral agents with potential for broad-spectrum activity.

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References