Commentary

What are the pros and cons of the use of host-targeted agents against hepatitis C?

CrossMark

Jean-Michel Pawlotsky *

National Reference Center for Viral Hepatitis B, C and D, Department of Virology, Hôpital Henri Mondor, Université Paris-Est, Créteil, France
INSERM U955, Créteil, France

ABSTRACT

Hepatitis C virus (HCV) therapy is living a revolution. Host-targeted agents (HTAs) block HCV production by interacting with host cell components. Because they target conserved host proteins, not variable viral proteins, HTAs have the potential for pangenotypic antiviral activity and a high barrier to resistance. Only two HTAs have reached clinical development, including specific inhibitors of cyclophilin A peptidyl-prolyl *cis/trans* isomerase activity and antagonists of microRNA-122. Cyclophilin inhibitors have proven to be relatively well tolerated and can be confidently used as backbones of all-oral, interferon-free regimens. In addition, HTAs such as cyclophilin inhibitors offer opportunities for "panviral" approaches when they target mechanisms common to viruses of the same or different families. This article forms part of a symposium in Antiviral Research on "Hepatitis C: next steps toward global eradication."

1. Introduction

Hepatitis C virus (HCV) therapy is living a revolution, with four already approved new antiviral agents and dozens at Phase 2 or 3 clinical developmental stages. New HCV drugs split into two groups: direct-acting antivirals (DAAs), that target viral actors of the HCV lifecycle, and host-targeted agents (HTAs), that block HCV production by interacting with host cell components (Pawlotsky, 2013). For many years, only HTAs were available for the treatment of chronic hepatitis C. They included interferon-α (IFN-α), which triggers an intracellular cascade of events that, in turn, activate a number of nonspecific antiviral cellular effectors, and ribavirin, a drug that accelerates infected cell clearance or cure when HCV production is potently inhibited through yet unknown mechanisms (Chevaliez and Pawlotsky, 2009; Thomas et al., 2013; Feld, in press; Koh and Liang, in press). More recently, HTAs targeting host cell components involved in complex interactions with viral proteins that are essential to the HCV lifecycle have been developed.

2. The hepatitis C virus lifecycle

The HCV lifecycle starts with receptor binding at the surface of hepatocytes. HCV entry into cells is pH-dependent and related to clathrin-mediated endocytosis. It is followed by a fusion step within an acidic endosomal compartment. Decapsidation of viral nucleocapsids liberates free positive-strand genomic RNAs in the cell cytoplasm, where they serve, together with newly synthesized RNAs, as messenger RNAs for synthesis of the HCV polyprotein. The polyprotein is targeted to the endoplasmic reticulum membrane where co- and post-translational processing by host cell peptidases, the NS2 autocatalytic protease and the NS3-4A serine protease results in the generation of the 3 structural proteins (C or core, E1 and E2), the p7 viroporin, and the 6 nonstructural proteins (NS2, NS3, NS4A, NS4B, NS5A and NS5B). Replication is catalyzed by the HCV RNA-dependent RNA polymerase (RdRp) or NS5B protein. The NS5A protein and the NS3 helicase-NTPase domain of the NS3 protein play an important regulatory role in virus replication, whereas NS4B serves as a membrane anchor for the replication complex and plays an important role in membrane rearrangements leading to its formation. Viral particle formation is initiated by the interaction of the core protein with genomic RNA. HCV then uses the lipoprotein production pathway to generate mature viral particles and export them (Lindenbach, 2013; Lohmann, 2013; Niepmann, 2013; Zeisel et al., 2013).

3. Host factors involved in the HCV lifecycle

Host cell proteins are involved at every step of the HCV lifecycle (see Table 1). HCV entry is mediated by several cell surface and tight junction proteins, including the low-density lipoprotein

(LDL) receptor, CD81, the scavenger receptor B1, claudin-1, occludin and the epidermal growth factor receptor (Zeisel et al., 2013).

Translation uses the cellular ribosomal machinery and three eukaryotic initiation factors (eIF): eIF2, eIF3 and eIF5. It also recruits noncanonical RNA-binding proteins, including the La protein, NSAP1 (NS1-associated protein 1), hnRNP (heterogeneous nuclear ribonucleoproteins) L and D, IMP-1 (insulin-like growth factor 2 mRNA binding protein 1), gemin-5, "like-Sm" protein LSm1-7, and PCBP2 (poly(rC) binding protein 2), which interact with the viral genome and with each other (Niepmann, 2013). MicroRNA-122 (miR-122) fixation to the 5′ noncoding region of the genome appears to stimulate translation by stabilizing the RNA. The contribution of the 3′ end of the viral genome to translation appears to be mediated by two cell proteins, the NFAR (nuclear factors associated with dsRNA) protein complex and IMP-1 (Niepmann, 2013).

A number of host cell proteins have been shown to contribute to the formation of the replication complex (or membranous web), such as PI4KIIIα (lipid kinase phosphatidylinositol 4-kinase III alpha), FBL2 (F-box and leucine rich repeat protein 2), host cell lipids and lipid droplets (Lohmann, 2013). A large number of viral proteins are involved in RNA synthesis, which is catalyzed by the viral RdRp under the control of the viral NS5A protein. They have been shown to interact with both viral proteins and to be required for viral replication. They include cyclophilin A, miR-122, hVAP-A (human VAMP-associated protein A) and its isoform hVAP-B, and cellular RNA-binding proteins (Lohmann, 2013).

HCV particle assembly shares numerous features with the VLDL/LDL assembly pathway, interacting with a number of apolipoproteins, in particular apoB and apoE, while MTP (microsomal triglyceride transfer protein) plays a major role in VLDL secretion and viral particle production (Lindenbach, 2013). In addition, HCV uses the ESCRT (endosomal-sorting complex required for transport) pathway, recycling endosomal compartments and a number of host cell factors (e.g. phospholipase A2, the µ1 subunit of AP2M1 [clathrin adapter protein complex 2], annexin A2, and stress granule proteins) for assembly and budding from the cytoplasm. Viral particles then pass through the Golgi apparatus in a VAMP-dependent manner (Lindenbach, 2013).

4. Host-targeted approaches

Blocking the interaction of any of these cell components with the HCV lifecycle results in an efficient blockade of viral production, as shown *in vitro* with specific inhibitors or silencing RNAs. Thus, numerous host-targeted approaches can theoretically be envisaged to inhibit HCV replication. However, only two reached clinical development thus far, including specific inhibitors of cyclophilin A peptidyl-prolyl *cis/trans* isomerase activity and antagonists of miR-122. Both classes of compounds reduced viral replication by several logs when administered to infected patients in Phase Ib and II clinical trials (Flisiak et al., 2008; Janssen et al., 2013). Drugs that interact with the lipid metabolism, such as statins, have been shown to inhibit the *in vitro* replication of HCV by blocking the late steps of the HCV lifecycle. The potential clinical benefit of statins in HCV-infected patients is at best modest (O'Leary et al., 2007; Patel et al., 2011; Zhu et al., 2013). Given the results of current and future anti-HCV therapies, it is unlikely that other host-targeted approaches will reach clinical development in this indication.

5. The place of host-targeted agents in HCV therapy

The vast majority of new HCV drugs in development are DAAs. They include inhibitors of the NS3-4A protease, nucleoside/nucleotide analogue inhibitors of the HCV RdRp, non-nucleoside inhibitors of RdRp, and NS5A inhibitors (Pawlotsky, 2013). These compounds are potent. Most first-generation drugs have a low barrier to resistance, with the notable exception of nucleoside/nucleotide analogues. Second-generation drugs with a higher barrier to resistance are currently being developed (Pawlotsky, 2013). What is, in this context, the place of host-targeted agents?

To cure HCV infection, a drug regimen must combine antiviral potency and a high barrier to resistance that ensures that inhibition of virus production is sustained over the full treatment period. When this is the case, infected cells progressively eliminate the RNA genomes they contain until no more cells are infected at the time treatment is withdrawn. Cyclophilin A inhibitors were shown to potently inhibit viral replication, both *in vitro* and *in vivo* (Flisiak et al., 2008; Paeshuyse et al., 2006). Because they target conserved host proteins, not variable viral proteins, both cyclophilin A inhibitors and miR-122 antagonists have pangenotypic antiviral activity and a high barrier to resistance (Coelmont et al., 2009; Flisiak et al., 2008; Lanford et al., 2010; Paeshuyse et al., 2006; Puyang et al., 2010). No resistance has been reported thus far with miR-122 antagonists *in vivo*. However, viral polymorphisms associated with resistance to miR-122 antagonism have been reported in a recent *in vitro* study (Li et al., 2011). Whether such variants naturally exist in infected patients is unknown. Amino acid substitutions in viral proteins are hardly selected by cyclophilin A inhibitors after numerous passages *in vitro* (Coelmont et al., 2009; Flisiak et al., 2008; Lanford et al., 2010; Paeshuyse et al., 2006; Puyang et al., 2010). They are essentially located in the NS5A protein sequence, confer minor reduction in drug susceptibility *in vitro*, and do not appear to play a role in cyclophilin inhibitor treatment failures *in vivo*. HTAs thus represent ideal "backbones" for pangenotypic drug combinations with potent antiviral activity and a high barrier to resistance, both for first-line use in treatment-naïve patients or

Table 1
Host factors that are known to be involved in the hepatitis C virus lifecycle.

Hepatitis C virus lifecycle step	Host factors known to be involved
Entry into cells	LDL receptor
	CD81 molecule
	Scavenger receptor B1
	Claudin-1
	Occludin
	Epidermal growth factor receptor
Viral polyprotein translation	Cellular ribosomal machinery
	eIF2, eIF3 and eIF5
	La protein
	NSAP1
	hnRNP L and D
	IMP-1
	Gemin 5
	"like Sm" protein LSm1-7
	PCBP2
	miR-122
	NFAR
Viral genome replication	Host cell lipids and lipid droplets
	PI4KIIIα
	FBL2
	Cyclophilin A
	miR-122
	hVAP-A and hVAP-B
	cellular RNA-binding proteins
Virion assembly and release	Apolipoproteins B and E
	MTP
	ESCRT
	Recycling endosomal compartments
	Phospholipase A2
	µ1 subunit of AP2M1
	Annexin 2
	Stress granule proteins
	VAMP

rescue therapy in patients who failed on prior IFN-containing or IFN-free regimens.

Host-targeted anti-HCV approaches however have caveats. In theory, because they target host cellular functions that may be essential to cell survival, they can be toxic. The peptidyl-prolyl cis/trans isomerase activity of cyclophilins catalyzes the isomerization of peptide bonds at proline residues and facilitates protein folding. However, mice knocked-out for cyclophilins are viable, and they even seem to be protected against lethal heart ischemia–reperfusion injury (Belaidi et al., 2013). In clinical practice, the administration of alisporivir, a cyclophilin A inhibitor derived from cyclosporine A currently in Phase II clinical development, was associated with mild to moderate hyperbilirubinemia, that was dose-dependent and reversible upon treatment withdrawal. Hyperbilirubinemia was mixed, due to inhibition of the uptake unconjugated bilirubin transporters organic anion transporter bilirubin OATP1B1 and OATP1B3 and of conjugated bilirubin efflux transporter canalicular multispecific organic anion transporter 1 MRP2, without inhibition of glucuronide conjugation (Flisiak et al., 2008; Pawlotsky et al., 2012b). Alisporivir administration was also associated with hypertriglyceridemia (Pawlotsky et al., 2012b), which may have aggravated acute pancreatitis cases that occurred in combination with IFN-α (including one fatal case). For this reason, the clinical efficacy of alisporivir is currently being studied as part of IFN-free combinations only. No side effects were observed over 2 weeks of administration of miravirsen, an miR-122 antagonist (Janssen et al., 2013). However, concerns have been raised as to the long-term effects of inhibiting miR-122, because low miR-122 levels in hepatocytes have been reported to be associated with hepatocellular carcinoma (Tsai et al., 2012).

Theoretically, any approach targeting host cell functions involved in the HCV lifecycle can lead to expected or unexpected side effects. It must be noted, however, that unexpected, sometimes serious adverse events are not specific for HTAs. Numerous side effects have been reported with inhibitors from each of the different classes of HCV DAAs. The development of several compounds was halted early because of serious adverse events, including fatal cases. The large-scale use of the two first-generation protease inhibitors telaprevir and boceprevir in combination with pegylated IFN-α and ribavirin was associated with frequent side effects, with particular toxicity in patients with advanced liver disease (Hezode et al., 2013). Finally, not all DAAs currently in clinical development are entirely safe. Overall, over the past 10 years of HCV drug development, HTAs directly targeting host cell components involved in the HCV lifecycle did not appear to be substantially more toxic than DAAs.

The high barrier to resistance of HTAs means that, in contrast with DAAs, their antiviral effect is not or poorly influenced by viral genetic polymorphisms. However, HTAs are exposed to host genetic polymorphisms that may alter their ability to block their target protein function. In clinical practice, 10 to 15% of patients have a suboptimal antiviral response to alisporivir (Pawlotsky et al., 2012a). The underlying mechanism is unknown. More work is needed to understand the genetic basis of primary non-response to host-targeted approaches.

Drug-drug interactions are possible with HTAs, like with DAAs, depending on the metabolic pathways they use. Their mode of administration can also be an issue. Cyclophilin inhibitors are administered orally, but the miR-122 antagonist miravirsen must be injected, a clear disadvantage at the era of all-oral therapies (Janssen et al., 2013). Future host-targeted approaches will need to be orally administered.

Where will host-targeted approaches fit in the HCV drug armamentarium? Currently, the only viable approach seems to be cyclophilin inhibition. Indeed, cyclophilin inhibitors can be confidently used as backbones of all-oral, IFN-free regimens because of their potency, their pangenotypic activity, their high barrier to resistance and their good tolerance in the absence of IFN-α co-administration. A recent study showed that alisporivir has synergistic effects in vitro with different families of DAAs (Garcia-Rivera et al., 2013). Thus, drug regimens combining a cyclophilin inhibitor with a protease inhibitor, an RdRp inhibitor and/or an NS5A inhibitor with or without ribavirin are credible and will likely enter clinical evaluation soon. Whether or not other host-targeted approaches should be investigated for the treatment of HCV infections is debatable. In this respect, it is interesting to note that the drug industry currently disinvests from new HCV drug screening and optimization, with the belief that enough drugs will be on the market soon to fulfill the clinical needs.

6. The place of host-targeted agents in therapy of other viral infections

HTAs, because they target host factors involved in the viral lifecycle and not the virus itself, have the potential for broader indications than DAAs which, by nature, are active against HCV only. Cyclophilin inhibitors were shown to inhibit the replication of hepatitis B virus, HIV and coronaviruses (Frausto et al., 2013; Phillips et al., 2012; Tanaka et al., 2013). Host-targeted antiviral approaches offer the opportunity of "panviral" antiviral approaches when they target mechanisms that are common to viruses that belong to the same or different families. Given the number of viral infections that represent important public health problems for which no specific antiviral compounds exist or are likely to be developed in the short- to mid-term, research on host-targeted approaches to combat viral infections should be encouraged. What we learnt from 20 years of HCV research and drug development may be particularly useful in this respect, making HCV an invaluable model for future antiviral drug development.

Conflict of interest disclosure

References

Belaidi, E., Decorps, J., Augeul, L., Durand, A., Ovize, M., 2013. Endoplasmic reticulum stress contributes to heart protection induced by cyclophilin D inhibition. Basic Res. Cardiol. 108, 363.

Chevaliez, S., Pawlotsky, J.M., 2009. Interferons and their use in persistent viral infections. Handb. Exp. Pharmacol., 203–241.

Coelmont, L., Kaptein, S., Paeshuyse, J., Vliegen, I., Dumont, J.M., Vuagniaux, G., Neyts, J., 2009. Debio-025, a cyclophilin binding molecule, is highly efficient in clearing hepatitis C virus (HCV) replicon-containing cells when used alone or in combination with specifically targeted antiviral therapy for HCV (STAT-C) inhibitors. Antimicrob. Agents Chemother. 53, 967–976.

Feld, J., in press. The beginning of the end: What is the future of interferon therapy for chronic hepatitis C? Antiviral Res.

Flisiak, R., Horban, A., Gallay, P., Bobardt, M., Selvarajah, S., Wiercinska-Drapalo, A., Siwak, E., Cielniak, I., Higersberger, J., Kierkus, J., Aeschlimann, C., Grosgurin, P., Nicolas-Metral, V., Dumont, J.M., Porchet, H., Crabbe, R., Scalfaro, P., 2008. The cyclophilin inhibitor Debio-025 shows potent anti-hepatitis C effect in patients coinfected with hepatitis C and human immunodeficiency virus. Hepatology 47, 817–826.

Frausto, S.D., Lee, E., Tang, H.L., 2013. Cyclophilins as modulators of viral replication. Viruses-Basel 5, 1684–1701.

Garcia-Rivera, J., Chatterji, U., Gallay, P., 2013. Cyclophilin inhibitor alisporivir (ALV) combinations with direct acting antivirals reveal strong synergistic anti-HCV effects. J. Hepatol. 58, S469-S469.

Hezode, C., Fontaine, H., Dorival, C., Larrey, D., Zoulim, F., Canva, V., de Ledinghen, V., Poynard, T., Samuel, D., Bourliere, M., Zarski, J.P., Raabe, J.J., Alric, L., Marcellin, P., Riachi, G., Bernard, P.H., Loustaud-Ratti, V., Metivier, S., Tran, A., Serfaty, L., Abergel, A., Causse, X., Di Martino, V., Guyader, D., Lucidarme, D., Grando-Lemaire, V., Hillon, P., Feray, C., Dao, T., Cacoub, P., Rosa, I., Attali, P., Petrov-Sanchez, V., Barthe, Y., Pawlotsky, J.M., Pol, S., Carrat, F., Bronowicki, J.P., 2013.

Triple therapy in treatment-experienced patients with HCV cirrhosis in a multicentre cohort of the French Early Access Programme (ANRS CO20-CUPIC) – NCT01514890. J. Hepatol. 59, 434–441.

Janssen, H.L., Reesink, H.W., Lawitz, E.J., Zeuzem, S., Rodriguez-Torres, M., Patel, K., van der Meer, A.J., Patick, A.K., Chen, A., Zhou, Y., Persson, R., King, B.D., Kauppinen, S., Levin, A.A., Hodges, M.R., 2013. Treatment of HCV infection by targeting microRNA. N. Engl. J. Med. 368, 1685–1694.

Koh, C., Liang, J., in press. What is the future of ribavirin therapy for hepatitis C? Antiviral Res.

Lanford, R.E., Hildebrandt-Eriksen, E.S., Petri, A., Persson, R., Lindow, M., Munk, M.E., Kauppinen, S., Orum, H., 2010. Therapeutic silencing of microRNA-122 in primates with chronic hepatitis C virus infection. Science 327, 198–201.

Li, Y.P., Gottwein, J.M., Scheel, T.K., Jensen, T.B., Bukh, J., 2011. MicroRNA-122 antagonism against hepatitis C virus genotypes 1–6 and reduced efficacy by host RNA insertion or mutations in the HCV 5′ UTR. Proc. Natl. Acad. Sci. USA 108, 4991–4996.

Lindenbach, B.D., 2013. Virion assembly and release. Curr. Top. Microbiol. Immunol. 369, 199–218.

Lohmann, V., 2013. Hepatitis C virus RNA replication. Curr. Top. Microbiol. Immunol. 369, 167–198.

Niepmann, M., 2013. Hepatitis C virus RNA translation. Curr. Top. Microbiol. Immunol. 369, 143–166.

O'Leary, J.G., Chan, J.L., McMahon, C.M., Chung, R.T., 2007. Atorvastatin does not exhibit antiviral activity against HCV at conventional doses: a pilot clinical trial. Hepatology 45, 895–898.

Paeshuyse, J., Kaul, A., De Clercq, E., Rosenwirth, B., Dumont, J.M., Scalfaro, P., Bartenschlager, R., Neyts, J., 2006. The non-immunosuppressive cyclosporin Debio-025 is a potent inhibitor of hepatitis C virus replication in vitro. Hepatology 43, 761–770.

Patel, K., Lim, S.G., Cheng, C.W., Lawitz, E., Tillmann, H.L., Chopra, N., Altmeyer, R., Randle, J.C., McHutchison, J.G., 2011. Open-label phase 1b pilot study to assess the antiviral efficacy of simvastatin combined with sertraline in chronic hepatitis C patients. Antiviral Ther. 16, 1341–1346.

Pawlotsky, J.M., 2013. Treatment of chronic hepatitis C: current and future. Curr. Top. Microbiol. Immunol. 369, 321–342.

Pawlotsky, J.M., Sarin, S.K., Foster, G., Peng, C.Y., Rasenack, J., Flisiak, R., Piratvisuth, T., Wedemeyer, H., Chuang, W.L., Zhang, W.M., Naoumov, N.V., 2012a. Alisporivir plus ribavirin is highly effective as interferon-free or interferon-add-on regimen in previously untreated HCV-GT2 or GT3 patients: SVR12 results from VITAL-1 Phase 2B study. J. Hepatol. 56, S553–S553.

Pawlotsky, J.M., Sarin, S.K., Foster, G.R., Peng, C.Y., Rasenack, J., Flisiak, R., Piratvisuth, T., Wedemeyer, H., Chuang, W.L., Zhang, W., Naoumov, N.V., 2012b. Alisporivir plus ribavirin achieves high rates of sustained HCV clearance (SVR24) as interferon (IFN)-free or IFN-add-on regimen in treatment-naive patients with HCV GT2 or GT3: Final results from VITAL-1 study. Hepatology 56, 309A–310A.

Phillips, S., Chokshi, S., Riva, A., Naoumov, N.V., 2012. Alisporivir-induced inhibition of cellular cyclophilins disrupts hepatitis B virus (HBV) replication in vitro and is synergistic in combination with direct antiviral targeting HBV DNA polymerase. J. Hepatol. 56, S199–S200.

Puyang, X., Poulin, D.L., Mathy, J.E., Anderson, L.J., Ma, S., Fang, Z., Zhu, S., Lin, K., Fujimoto, R., Compton, T., Wiedmann, B., 2010. Mechanism of resistance of hepatitis C virus replicons to structurally distinct cyclophilin inhibitors. Antimicrob. Agents Chemother. 54, 1981–1987.

Tanaka, Y., Sato, Y., Sasaki, T., 2013. Suppression of coronavirus replication by cyclophilin inhibitors. Viruses-Basel 5, 1250–1260.

Thomas, E., Ghany, M.G., Liang, T.J., 2013. The application and mechanism of action of ribavirin in therapy of hepatitis C. Antiviral Chem. Chemother. 23, 1–12.

Tsai, W.C., Hsu, S.D., Hsu, C.S., Lai, T.C., Chen, S.J., Shen, R., Huang, Y., Chen, H.C., Lee, C.H., Tsai, T.F., Hsu, M.T., Wu, J.C., Huang, H.D., Shiao, M.S., Hsiao, M., Tsou, A.P., 2012. MicroRNA-122 plays a critical role in liver homeostasis and hepatocarcinogenesis. J. Clin. Invest. 122, 2884–2897.

Zeisel, M.B., Felmlee, D.J., Baumert, T.F., 2013. Hepatitis C virus entry. Curr. Top. Microbiol. Immunol. 369, 87–112.

Zhu, Q., Li, N., Han, Q., Zhang, P., Yang, C., Zeng, X., Chen, Y., Lv, Y., Liu, X., Liu, Z., 2013. Statin therapy improves response to interferon alfa and ribavirin in chronic hepatitis C: a systematic review and meta-analysis. Antiviral Res. 98, 373–379.