Recent advances in the management of viral hepatitis

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ABSTRACT While viral hepatitis is a global problem its prevalence in the UK is often underestimated. Chronic infection with the hepatitis B and/or C virus causes significant morbidity and mortality. New treatments that attenuate viral replication or induce immunity against infection have transformed the management of these conditions, but their effectiveness comes at some cost – both in financial terms and in the side-effect profile associated with treatment. Viral resistance promises to be an ongoing problem, particularly in patients who have an inadequate response to antiviral therapy or are non-adherent with treatment protocols. This article explores new developments in the treatment of chronic hepatitis B and C infection, and describes current protocols for managing patients with these conditions.

KEYWORDS Hepatitis B, Hepatitis C, liver disease, protease inhibitors, nucleos(t)ide analogues, polymerase inhibitors

DECLARATION OF INTERESTS No conflict of interests declared.

INTRODUCTION

Viral hepatitis is thought to kill more than a million people each year as a result of liver failure or cancer. While the UK is considered to have a low population prevalence of chronic viral hepatitis, the number of patients in urban centres is increasing – despite the incidence of acute viral hepatitis remaining relatively static. The explanation lies in patterns of population migration; the number of individuals born in countries with a high prevalence of chronic viral hepatitis who subsequently move to UK cities has steadily increased during the past 25 years, changing the frequency of these diseases within urban populations in the process. The rise in numbers of patients infected with hepatitis B and C has inevitably led to an increase in complications of chronic disease, namely liver failure and malignancy. The challenge is to identify at-risk individuals before the complications of chronic infection become established.

HEPATITIS B

Despite the existence of a safe and effective vaccine, almost one in three people across the world have been infected with the hepatitis B virus. The World Health Organization estimates that a total of 350 million people are chronically infected and an estimated 600,000 persons die each year as a consequence. The majority of patients infected in adulthood will develop immunity to the virus, but vertical transmission from mother to child or exposure to the virus in early childhood is likely to result in chronic infection. In these children, chronic hepatitis B virus (HBV) infection is associated with a state of relative immune tolerance until young adulthood, when, as a result of factors that are poorly understood, the immune system attempts to eradicate the virus.

In patients who mount a successful immune response the viral titres fall and are associated with loss of the
hepatitis B e-antigen (HBeAg-negative), a secreted product of the HBV nucleocapsid gene. The majority of these individuals maintain a low state of viral replication and are unlikely to develop significant liver disease, but a significant proportion of patients fail to complete (or maintain) e-antigen seroconversion and are destined to go through iterative cycles of abortive clearance. The ensuing chronic inflammation, hepatocyte death and fibrosis predisposes to cirrhosis and/or hepatocellular cancer (Figure 1). Up to a third of HBeAg-negative individuals either fail to adequately repress viral replication or experience successional viral reactivation after HBeAg seroconversion (particularly patients with genotype B, C or D infection) as a result of mutations within the viral core or basal promoter sequence. These patients, often Mediterranean or East Asian males in their fourth or fifth decade, are at particular risk of developing cirrhosis if signs of viral reactivation are missed.

Population studies such as Risk Evaluation of Viral Load Elevation and Associated Liver disease/cancer–hepatitis B virus (REVEAL) have enabled us to characterise those individuals at increased risk of developing cirrhosis or cancer. This prospective multicentre cohort study, based in Taiwan, amassed almost 43,000 person-years of follow-up in HBV-positive individuals. While the majority of these patients were HBeAg-negative, the likelihood of developing cirrhosis was increased in patients with longstanding infection, particularly those individuals with high levels of viral replication. Patients at highest risk had viral loads >10^10 copies/ml, where rates of cirrhosis over the 13 years of follow-up were as high as 36%; three-quarters of the cases of cirrhosis seen occurred in the 44% of individuals (n=1,563) whose viral titres were ≥10,000 copies/ml (~2,000 IU/ml). Similarly, the risk of malignancy increased with time and was greatest in patients with high levels of viral replication (>10^7 copies/ml). The risk of developing cirrhosis or hepatocellular carcinoma (HCC) was much lower in patients with levels of viral replication <2,000 IU/ml.

Viral titre is not the only determinant of cancer risk; exposure to mutagens and carcinogens such as aflatoxin and dietary iron are important environmental factors, as is the co-existence of other diseases such as human immunodeficiency virus (HIV)/hepatitis C virus (HCV) co-infection or obesity. The viral genotype is important. Genotype-C HBV is associated with delayed HBeAg seroconversion, an increased viral load and more advanced liver fibrosis, as well as an increased lifetime risk of HCC. Mutations within the viral genome also modulate the outcome of chronic infection, as the basal-core promoter (BCP) and pre-S deletion mutations are both associated with an increased risk of malignant change. In fact, multivariate analysis, including viral genotype, BCP and pre-core sequence analysis, shows that the increased risk for genotype-C infection may be dependent on the emergence of specific viral mutations.

Guidelines for therapy in chronic hepatitis B infection
A number of long-term follow-up studies have demonstrated that the loss of the HBeAg and a reduction in viral titre is associated with increased survival and a reduced risk of developing HCC. If lower levels of viral replication are associated with a better clinical outcome, it seems reasonable to assume that treating patients with high levels of viral activity may prevent the complications of chronic infection. The ability to routinely measure HBV deoxyribonucleic acid (DNA) titres allows us to identify patients at risk of developing significant end-organ disease. The most recent guidelines for the investigation of patients with chronic hepatitis B suggest that any patient with an abnormal alanine transaminase (ALT) or HBV DNA >2,000 IU/ml (or both) should undergo liver biopsy to establish whether they have evidence of liver fibrosis.

This presents a challenge for clinicians, as it is difficult (and sometimes unnecessary) to biopsy everyone who meets these criteria. Viral titres fluctuate, some patients are immuno-tolerant and therefore have no evidence of liver disease despite high levels of viral replication and others have abnormal liver function tests despite low viral titres due to other disease processes such as non-alcoholic fatty liver disease: only a very small proportion of patients with consistently normal ALT and minimally elevated viral titres will have clinically significant fibrosis. In such cases a policy of watchful waiting allied with the use of non-invasive markers of liver fibrosis would seem reasonable unless there is a strong pedigree of HBV-related liver cancer, where early treatment and regular ultrasound surveillance might be preferable. Older patients are at increased risk of malignancy and cirrhosis, and a lower threshold for biopsy is recommended in these cases to establish the degree of architectural distortion and the need for treatment.

Non-invasive markers of fibrosis (serum markers and/or transient elastography) allied with a high quality ultrasound examination of the liver have transformed the management of viral hepatitis and help to identify patients with fibrosis who will go on to develop significant disease. Ultrasound of the edge of the left lobe of the liver has a strong positive predictive value for identifying fibrotic change, while transient elastography is a powerful tool that can determine normal or significantly fibrotic parenchyma without recourse to biopsy. Liver biopsy should be used for patients in whom the diagnosis remains in doubt, although clinicians need to be aware that fibrosis in chronic viral hepatitis is not uniform and can be subject to considerable spatial variation.

It is important that patients with evidence of active viral replication remain under follow-up in specialist clinics where their disease can be monitored. Patients with established cirrhosis or evidence of clinically significant fibrosis (Metavir ≥2) in the presence of active
viral replication should receive antiviral therapy to prevent disease progression and minimise the risk of long-term complications.

**Therapeutic options in chronic hepatitis B**

Therapeutic options for the treatment of chronic hepatitis B have improved over the past ten years (Table 1). Seven therapies are approved, including two formulations of interferon and five orally administered nucleos(t)ide analogues. Pegylated interferon (Peg-IFN) remains the preferred option of many physicians as first-line therapy in HBeAg-positive infection, where the aim of therapy is to achieve suppression of viral replication and/or HBsAg seroconversion. Seroconversion is seen in up to 30% of patients during a 48-week course of therapy and HBeAg seroconversion rates are increased for up to two years following treatment.10

The response to IFN is dependent on virological factors such as eAg titre, viral genotype and mutations in the BCP region,12,13 as well as the host immune response, but IFN has the advantage of not inducing viral resistance. Some studies suggest that the response to IFN therapy can be predicted by measuring the fall in HBeAg and/or HBsAg titre during therapy. Patients with a significant early fall in HBsAg titre are more likely to maintain a virological response and achieve HBsAg seroconversion, possibly because the HBsAg titre acts as a surrogate marker of the level of archived intrahepatic HBV covalently closed circular DNA.14 It is too early to tell whether quantitative HBsAg measurement will become a standard part of IFN therapy, but HBsAg titres appear to have a better predictive value than the level of HBV DNA. In the past few years there has been renewed interest in using Peg-IFN in HBeAg-negative chronic hepatitis B, particularly in patients who achieve a significant reduction in HBsAg titres during the first six months of therapy.15

For patients who fail to respond, or are unsuitable for IFN therapy (e.g. cirrhotics), nucleos(t)ide analogues provide an alternative form of therapy, both for HBeAg-positive and -negative disease. These drugs substantially reduce viral activity by inhibiting HBV reverse transcription and DNA replication. A new generation of nucleos(t)ide analogues, capable of maintaining prolonged suppression of viral replication, reward the clinician with substantial improvements both in the grade of inflammation and the stage of fibrosis: over time they may even reduce the risk of HCC.16,17 Older nucleos(t)ide analogues are vulnerable to viral resistance, particularly lamivudine, which has been associated with rates of resistance of up to 30% after 12 months of therapy. Lamivudine is no longer recommended for use in nucleos(t)ide-naive patients, although it may still have a role as a prophylactic agent in older HBSAg-positive patients undergoing chemotherapy or immunosuppressive therapies. In short-term follow-up studies, combinations of Peg-IFN and lamivudine were not shown to be superior to monotherapy with either agent alone.18,19

Patients with liver fibrosis as a consequence of HBeAg-negative disease due to the emergence of pre-core or BCP mutants should be treated with nucleos(t)ide analogues once they develop clinically significant fibrosis (≥Metavir F2). These drugs are powerful inhibitors of HBV replication and the newer, highly potent nucleos(t)ide analogues achieve complete virological suppression in HBeAg-negative disease in 80–90% of patients within a year of commencing therapy.20,21 Entecavir and/or tenofovir can be used in patients with decompensated cirrhosis because of their high genetic barrier to viral breakthrough, and both have been demonstrated to prevent viral reactivation following liver transplantation where they are used as prophylactics.22,23

The Achilles heel of nucleos(t)ide analogues lies in the potential for viral resistance. While rates of resistance are low in treatment-naïve patients treated with the newer, more potent nucleos(t)ide analogues, patients treated with (or exposed to) older agents commonly develop viral resistance, particularly if complete suppression of viral replication is not achieved. In such patients appropriate combinations of antiviral agents, chosen according to the resistance profile of the virus, and strict concordance with therapy will often restore suppression of viral replication. The response to antiviral therapy should be reassessed at six or 12 months after the initiation of therapy to ensure the patient has achieved adequate levels of viral suppression.

**HEPATITIS C**

Hepatitis C affects 170 million people worldwide, of whom approximately 20% will develop cirrhosis with the attendant risks of HCC and/or liver failure.24 In North America and Europe, hepatitis C has become one of the most common indications for liver transplantation and, as the infected population ages, it is likely that the number of people presenting with complications of chronic infection will increase.

Hepatitis C is a single-stranded ribonucleic acid (RNA) virus with six genotypes. The 9kB open reading frame encodes a long polyprotein which is processed (during and after translation) into ten proteins that have structural and non-structural properties (Figure 2). Like the HIV

**TABLE 1 Treatment options for chronic hepatitis B infection**

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virus, hepatitis C maintains a high rate of replication using an error-prone polymerase which produces an immense number of virological variants. This ensures that the virus exists in man as a quasispecies, consisting of a dominant wild-type virus and a multitude of less efficient variants. While the HCV is a relatively inefficient infective particle, it has highly adapted mechanisms for subverting viral sensing and response mechanisms within host cells. The NS3/4a protease interferes with key signal transduction molecules such as Cardif and TRIF and impairs the innate detection of viral protein, while structural core proteins and the NS5 polymerase interfere with interferon signal transduction, counteracting the IFN response by preventing release of IFN-stimulated gene products that possess antiviral properties.

The past 20 years have seen significant developments in the treatment of hepatitis C. The advent of Peg-IFN and identification of ribavirin (RBV) as a synergistic antiviral agent have increased response rates in some viral genotypes to almost 80%. Nevertheless a significant proportion of patients, particularly those with characteristics that denote a more challenging treatment cohort (Table 2), fail to respond to current standard of care antiviral therapy. A large study of Peg-IFN and RBV therapy in treatment-naïve genotype 1-infected individuals recently reported sustained antiviral reponse (SVR) rates of only 38–40% with a 20–30% relapse rate in responders. Treatment is often poorly tolerated, and up to a third of patients require dose reductions, while 10% experience severe side effects that require a cessation of therapy.

As our understanding of viral kinetics has improved it has become possible to tailor the course of treatment according to patient characteristics and viral genotype. Patients with good treatment parameters, a low viral load and rapid response to antiviral agents can be treated with short courses of therapy, whereas slow responders, who are at high risk of relapse, benefit from extended treatment courses. Similarly patients who were previously considered too difficult to treat (compensated cirrhosis, post-transplant HCV, HIV co-infected) are being enrolled in treatment programmes, with encouraging results.

**Genetic predictors of response to antiviral therapy**

The response to infection with the HCV shows considerable inter-individual variation. While the majority of people develop a chronic form of infection, 30% of individuals will spontaneously clear the virus and develop a form of protective immunity that reduces the likelihood of reinfection. There is considerable racial variation in the response. Caucasians are five times more likely to spontaneously clear HCV infection than black African-Americans, while East Asians have the best response rates to exogenous IFN reported in the literature. This suggests that genetic elements contribute to an individual’s response to antiviral therapy. Our understanding of these processes has been improved by a series of studies that have identified single nucleotide polymorphisms (SNPs) in and around the gene that encodes IFN-l3 (IL28B) that associate with a favourable response to antiviral therapy.

The first studies to link IL28B and the response to antiviral treatment were generated from large cohorts of patients infected with genotype 1 HCV who had been treated with Peg-IFN and RBV. Genome-wide association studies (GWAS) performed in these treatment-naïve patients identified a number of genetic markers (SNPs) that were strongly associated with the response to antiviral therapy. Despite testing more than 500,000 SNPs across almost 90% of the genome, researchers found that a region on the long arm of chromosome 19, upstream of the IL28B gene, was most strongly associated with sustained viral response (SVR) following antiviral therapy. Caucasians with the favourable response...
genotype (C-C) of the rs12979860 SNP were five times more likely to achieve an SVR following antiviral therapy than patients with the minor T-allele (C-T or T-T). Three other studies have independently found polymorphisms in SNPs associated with the IL28B gene that predict the response to antiviral therapy. The SNPs are not functional; they act as positional markers in the genome and allow researchers to localise the region (or haplotype) that is responsible for the effect. Further analysis of the region around the SNPs has identified a risk haplotype characterised by an arginine substitution at amino acid position 70 (K70R) in the IL28B gene which is strongly associated with non-response to antiviral therapy.

Subsequent studies have shown that responder genotypes are associated with increased rates of spontaneous clearance of HCV and are found most commonly in those parts of the world associated with better response rates to antiviral therapy. The favourable response genotype is paradoxically associated with a higher baseline viral load and a more rapid response to antiviral therapy than patients who carry the minor alleles, suggesting that the IL28B genotype denotes a more interferon-responsive phenotype.

IL28B encodes IFNλ3, one of a group of type-3 IFNs which include IL29 (IFNλ-1) and IL28A (IFNλ-2). Dendritic cells and mononuclear cells are the major sources of type-3 IFNs, although hepatocytes can probably release IFNλ during infection; unlike type-1 interferon, IL28B is upregulated in hepatocytes of individuals infected with HCV and phase-1 trials of IFNλ-1 in treatment-naïve genotype 1-infected patients resulted in a significant fall in HCV RNA over four weeks. Commercial tests that identify the responder-genotype are available, but their use has yet to be established in routine clinical practice.

**Adaptation of existing therapies for hepatitis C**

Current antiviral therapy has changed very little in the past ten years. A number of new long-acting IFNs are in development, hoping to improve the pharmacodynamics of the IFN response and reduce dosing frequency. Albuferon, a recombinant IFNλ-2b fused to human albumin, is a long-acting IFN that can be given every fortnight. Other modified release systems are in varying stages of development; locteron is a fortnightly preparation of recombinant alpha interferon encapsulated in a biodegradable drug delivery system and several designer IFNs, including omega-IFN and an oral and injected form of genetically modified IFNλ, are being used in clinical trials.

One of the most common side effects of RBV therapy is haemolytic anaemia, which necessitates a dose reduction in almost 30% of patients. Ribavirin accumulates in erythrocytes where it is phosphorylated, depleting adenosine triphosphate (ATP) reserves, and ultimately leading to haemolysis. Interferon compounds matters by suppressing erythopoiesis, necessitating further reductions in RBV dose that may compromise the effectiveness of therapy. Taribavirin (viramidine) is a prodrug of RBV and is preferentially taken up by hepatocytes. A recent study using a weight-based regimen demonstrated similar SVR rates to standard RBV, but lower rates of anaemia, particularly in the first 12 weeks of therapy when RBV dosing is most critical.

A number of studies using the replicon system have suggested that nitazoxanide, a thiazolide anti-parasitic agent, has antiviral activity. The drug enhances the phosphorylation of PKR, a kinase that regulates cellular innate immune responses. A placebo-controlled study in genotype 4-positive patients receiving 500 mg twice daily of nitazoxanide for 24 weeks achieved undetectable viral RNA in 30% (7/23) of patients. A subsequent study in genotype 4-positive Egyptian patients compared nitazoxanide in combination with Peg-IFN with or without ribavirin and recorded a significantly increased SVR rate, even though end of treatment responses were similar in all arms. These interesting observations suggest that nitazoxanide may reduce relapse rates in G4 responders, although further studies are required before it can be considered part of routine antiviral therapy.

**Direct antiviral agents for CHC**

Current therapies for hepatitis C are expensive, often poorly tolerated and successful in just over half of patients treated; the side effects alone are responsible for 8–10% of patients being withdrawn from therapy. There is a need for new drugs that are able to eradicate the viral infection quickly (to reduce costs) with minimal toxicity. In recent years pharmaceutical companies have targeted the non-structural components of the HCV polyprotein, producing inhibitors of the NS3/4a serine protease and NS5b RNA-dependent RNA polymerase (RdRp) which promise to revolutionise our approach to anti-HCV therapy.

**NS3/4a protease inhibitors**

The NS3/4a protease is a key component of viral replication, responsible for the post-translational modification of the NS3–5 region of the HCV polyprotein as well as possessing a helicase function that allows it to unwind viral RNA. It can regulate host intracellular type I IFN pathways, interfering with viral sensing by preventing signal transduction via cleavage of key components of the interferon signalling pathway such as Cardif and TRIF and preventing activation of IRF-3. It is possible that potent inhibition of the NS3/4a protease may allow innate sensing pathways to recover, thus improving responses to exogenous interferon, although both assumptions remain hypothetical.

Telaprevir is a potent inhibitor of the NS3/4a protease whose antiviral effects are synergistically increased in combination with Peg-IFN and ribavirin. A phase 2 study...
A further study in patients who had previously failed antiviral therapy has demonstrated the potency of telaprevir combination therapy, achieving an SVR in 50% of patients treated with triple combination therapy compared to 14% of controls. This trial reaffirmed the importance of consolidation therapy after telaprevir withdrawal and the role of RBV in preventing virological breakthrough.

Viral resistance to protease inhibitors is often associated with a number of single- or double-point mutations in the catalytic region of the enzyme and is increased by low trough levels of drug. Telaprevir-resistant variants can be classified as low or high level, but all are less fit than the wild-type virus in vitro, with the most resistant A156V/T mutant being least fit. As one would expect, telaprevir-resistant variants appear to be cross-resistant in vitro. While the NS3/4a protease inhibitors are ineffective as monotherapy, their addition to existing therapies will facilitate shorter courses of treatment at the same time as significantly increasing SVR rates, albeit at some increased cost in terms of toxicity. It is also important to note that their activity is relatively genotype-dependent, and further studies will be needed to gauge their effectiveness in non-genotype 1 infection.

**Cyclophilin and NS5B RNA-dependent RNA-polymerase inhibitors**

The NS5B RNA-dependent RNA-polymerase (RdRp) plays a crucial role in viral replication, synthesising the complementary minus-strand RNA and subsequent genomic plus-strand from the minus strand template. The replicative activity of the enzyme is increased by direct binding to cyclophilin B, a host cell isomerase. Cyclosporin A has been known to inhibit this interaction for many years, and specific cyclophilin inhibitors are in various stages of development. Early phase I studies of these agents have shown promising results. The drugs are particularly important as they appear to be active against all genotypes and have no reported resistant viral mutants.

As mammals have no requirement for an RdRp, it should be possible for this enzyme to be selectively targeted without interfering with host cellular function. A variety of novel RdRp inhibitors are at advanced stages of development. While they do not produce the dramatic falls in viral RNA one associates with protease inhibitors, they appear to be more resistant to viral breakthrough, probably due to the low replicative capacity of drug-induced virological variants.

A recent study, combining a new RdRp inhibitor with danoprevir, a protease inhibitor, given to genotype 1 infected patients for up to 13 days as IFN-free combination therapy, achieved median reductions in viral load of 5 log₁₀ with no evidence of treatment-emergent resistance or dose reductions due to drug-related adverse events. This exciting study suggests that combining direct antiviral agents with a higher resistance barrier, such as RdRp inhibitors, with lower resistance but more potent drugs, such as protease inhibitors, will prevent resistance-associated virological breakthrough.

**Other agents**

A variety of new direct antiviral agents are being developed in addition to those mentioned here. Celgosivir, an α-glucosidase inhibitor, interferes with viral assembly and envelope protein folding by targeting a host enzyme involved in glycoprotein processing. While its effects are modest, it has the potential to be
active across genotypes and trials of combination therapy with IFN and ribavirin are under way. Anti-HCV antibodies are in development as entry inhibitors, as are specific molecules that target cell-surface receptors the HCV virus uses to enter the host cell such as SR-B1. Such agents are specifically targeted at preventing HCV recurrence at transplantation, although trials of HCV immunoglobulin therapy in the prevention of recurrence have been disappointing.

CONCLUSIONS

Recent developments in our understanding of viral replication and protein structure have produced new therapeutic agents that promise to revolutionise treatments for hepatitis B and hepatitis C infection. It is likely that combinations of direct antiviral agents will succeed in increasing rates of SVR using attenuated treatment courses. Side effects will remain a problem, and tolerability will be the limiting factor for many patients who receive these new treatments. Avoidance of drug-induced resistance will be an ongoing challenge. Nevertheless, these new treatments will offer hope to thousands of patients chronically infected with hepatitis B and C.

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