Hepatitis Research Review™

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In this issue:

Abbreviations used in this issue:

HBeAg = hepatitis B e antigen; **HBsAg** = hepatitis B surface antigen; **HBV** = hepatitis B virus; **HCV** = hepatitis C virus; **IFN** = interferon; **PegIFN** = pegylated interferon; **SOF+Peg-IFN+RBV** = sofosbuvir plus pegylated interferon and ribavirin; **SVR** = sustained virological response

Welcome to issue 18 of Hepatitis Research Review.

Promising data are reported from a phase II study showing high rates of sustained virological response (SVR) after 12 weeks of treatment with sofosbuvir plus pegylated interferon and ribavirin (SOF+Peg-IFN+RBV) in treatment-experienced patients with genotype 2 and 3 hepatitis C virus (HCV) infection, 55% of whom had compensated cirrhosis. Moreover, SOF+Peg-IFN+RBV was generally well tolerated, with low discontinuation rates and an adverse event profile consistent with Peg-IFN+RBV. This new regimen represents an important treatment option for these patients, who have historically exhibited suboptimal response rates to HCV treatment and for whom few treatment options exist.

Research from China reports that a hepatitis E vaccine provided protection against the virus for at least 4.5 years. The vaccine did not appear to be associated with any serious adverse events and at the time of the study analysis, there was no evidence that effectiveness was waning.

I hope you enjoy the selection in this issue and I welcome your comments and feedback.

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Saikosaponin b2 is a naturally occurring terpenoid that efficiently inhibits hepatitis C virus entry

Authors: Lin LT et al.

Summary: These researchers examined the effect of saikosaponins (SSa, SSb2, SSc, and SSd), derived from the root extract of *Bupleurum kaoi*, on the complete hepatitis C virus (HCV) life cycle (entry, RNA replication/translation, and particle production). They also evaluated antiviral activity against various HCV genotypes, clinical isolates, and infection of primary human hepatocytes. BK and the saikosaponins potently inhibited HCV infection at non-cytotoxic concentrations. These natural agents targeted early steps of the viral life cycle, and had little effect upon replication/translation, egress, and spread. SSb2 was particularly efficient at inhibiting early HCV entry, including neutralisation of virus particles, preventing viral attachment, and inhibiting viral entry/fusion. Binding analysis using soluble viral glycoproteins demonstrated that SSb2 acted on HCV E2. SSb2 inhibited infection by several genotypic strains and prevented binding of serum-derived HCV onto hepatoma cells. Treatment with SSb2 blocked HCV infection of primary human hepatocytes.

Comment: Much research over recent years focussing on mechanisms by which HCV gains entry into hepatocytes has demonstrated the importance of interactions between viral glycoproteins and various cellular entry factors and receptors, including glycosaminoglycans, the tetraspanin family protein member CD81, scavenger receptor class B type I, the low-density lipoprotein receptor and tight junction proteins including various claudins and occludin. This interesting study of saikosaponins, naturally occurring glycoside phytochemicals derived from the *Bupleurum kaoi* root whose structures resemble steroid hormones, found that saikosaponin b2 inhibits both HCV attachment and entry to hepatocytes. The findings support further preclinical testing, especially in combination with other therapies known to target other aspects of HCV pathobiology, such as viral replication.

Reference: J Hepatol. 2015;62(3):541-8

Abstract

Two-year treatment outcome of chronic hepatitis B infection treated with besifovir *vs*. entecavir: Results from a multicentre study

Authors: Yuen MF et al.

Summary: In this study, 89 treatment-naïve patients with chronic hepatitis B virus (HBV) infection receiving besifovir 90 mg (n=31), 150 mg (n=28) or entecavir 0.5 mg (n=30) were monitored for liver biochemistry, viral serology, HBV DNA levels, development of drug resistance mutations, and adverse events throughout 96 weeks of treatment. At end of treatment (week 96), HBV DNA levels had declined from baseline by a mean of 5.29, 5.15, and 5.67 log IU/mL with besifovir 90 mg, 150 mg and entecavir 0.5 mg, respectively (p>0.05); 80.7%, 78.6%, and 80% of patients, respectively, achieved undetectable HBV DNA (<20 IU/mL), ALT was normalised in 90.3%, 78.6%, and 93.3% of patients, while 20%, 21.4%, and 22.2%, respectively, experienced hepatitis B e antigen (HBeAg) loss (all p>0.05). One patient receiving besifovir 90 mg had a virological breakthrough due to drug non-compliance. No drug resistance mutations were detected. Ten patients had serious adverse events; these were deemed not related to study medication. Carnitine depletion was the most common side effect related to besifovir. Of the besifovir 90 mg and 150 mg treatment arms, 83.9% and 100% of patients, respectively, were prescribed carnitine supplements for low carnitine levels at any one time during follow-up. No patient had increased creatinine >0.5 mg/dL from baseline.

Comment: This issue includes two studies of novel nucleos(t)ide analogues developed to treat chronic HBV infection that are in differing stages of clinical development. In the first of these, the authors report on a two-year experience with besifovir, an acyclic nucleotide analogue, in comparison to entecavir in a roll-over analysis from a phase IIb open-label, multicentre randomised trial. Overall, rates of serum ALT normalisation and loss of HBeAg, along with degrees of decline in HBV DNA in peripheral blood, were not significantly different for the two antiviral agents. A potential drawback for besifovir therapy is the requirement for carnitine supplementation in order to prevent untoward effects such as hypoglycaemia and encephalopathy.

Reference: J Hepatol. 2015;62(3):526-32
Abstract

Twenty-eight day safety, antiviral activity, and pharmacokinetics of tenofovir alafenamide for treatment of chronic hepatitis B infection

Authors: Agaral K et al.

Summary: This study randomised 51 non-cirrhotic, treatment-naïve patients with chronic HBV to receive tenofovir alafenamide 8, 25, 40, or 120 mg, or tenofovir disoproxil fumarate 300 mg for 28 days. They were assessed during treatment for safety, antiviral response and pharmacokinetics. All patients completed study treatment and were followed-up for 4 weeks. Groups were generally well matched at baseline (67% male, 57% Asian, 53% HBeAg-negative, mean HBV DNA approximately 6.0 $\log_{10}$ IU/mL) with HBV genotypes reflective of the population. No serious or severe adverse events (grade 3/4) were reported. At week 4, mean decreases from baseline in serum HBV DNA were similar across the tenofovir alafenamide groups (–2.81, –2.55, –2.19, and –2.76 $\log_{10}$ IU/ml with tenofovir alafenamide 8, 25, 40, and 120 mg, respectively) and comparable to tenofovir disoproxil fumarate 300 mg (–2.68 $\log_{10}$ IU/mL). All groups had similar kinetics of viral decline. Tenofovir alafenamide pharmacokinetics were linear and dose-proportional; doses ≤25 mg were associated with ≥92% reductions in mean tenofovir area under the curve relative to tenofovir disoproxil fumarate 300 mg.

Comment: This phase Ib study assesses the short-term safety and antiviral efficacy of the nucleotide analogue inhibitor of HBV polymerase/reverse transcriptase, tenofovir alafenamide, a phosphonate prodrug of tenofovir disoproxil that has been shown to possess greater plasma stability than tenofovir disoproxil, an effect that promotes relatively higher intracellular levels. Tenofovir alafenamide was safe, well tolerated and demonstrated similar antiviral activity to tenofovir disoproxil, even when given at around one-tenth of the dose of the latter. Whether the theoretical advantage of lower-dose exposure made possible by the use of tenofovir alafenamide rather than tenofovir disoproxil will translate to any reduction in clinical adverse events during longer term exposure remains to be determined.

Reference: J Hepatol. 2015;62(3):533-40
Abstract

RESEARCH REVIEW
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Systems biological analyses reveal the hepatitis C virus (HCV)-specific regulation of hematopoietic development

Authors: Velazquez VM et al.

Summary: To examine the effects of chronic HCV on haematopoiesis, this investigation used a systems biology approach to study haematopoietic development in liver-resident cells expressing stem cell marker CD34. The analysis recruited patients with end-stage liver disease induced by chronic HCV, nonalcoholic steatohepatitis (NASH) and alcohol liver disease (ALD). Liver CD34+ cells were divided into two subsets, CD34+CD146+ and CD34+CD146–; hematopoietic function was restricted to CD34+CD146– cells. NASH and chronic HCV were associated with reduced liver CD34 frequencies compared with ALD, and this reduction correlated with viral load in the HCV cohort. Gene expression profiling and computational modelling was used to study the relationship between liver CD34+CD146+ and CD34+CD146– subsets and any effects of HCV and ALD on CD34 development. In comparison to CD34+CD146– cells, CD34+CD146+ cells had increased expression of endothelial cell genes, including *von Willebrand factor*, *VE-cadherin*, and *eNOS*. ALD and HCV diseases had only minimal effects upon gene expression. Importantly for CD34+CD146– cells, chronic HCV was associated with a distinct "imprint" of programs related to cell cycle, DNA repair, chemotaxis, development, and activation, with an emphasis on myeloid and B lymphocyte lineages. Under identical culture conditions, HCV CD34+CD146– cells demonstrated superior haematopoietic growth, colony formation, and diversification compared to CD34+CD146– cells in ALD and NASH diseases. Disease-associated effects on haematopoiesis were also evident in phenotypic alterations in the expression of CD14, HLA-DR, and CD16 by myeloid progeny cells.

Comment: This study demonstrates that the human liver is a source of haematopoietic cells and that local environmental cues such as inflammation and disease-specific factors influence their fate. In particular, HCV infection was shown to be associated with increased expression of genes associated with cell cycle activity, immune activation related to myeloid cells and B lymphocytes and haematopoietic development in liver-resident CD34+CD146– cells, but not in CD34+CD146+ cells. The haematopoietic potential of CD34+CD146– cells exposed to HCV was substantially more pronounced than that of CD34+CD146– cells from the livers of patients with alcohol-related and non-alcoholic fatty liver-related liver disease. This analysis is among the first to elucidate disease-specific patterns of haematopoietic development in adult peripheral organs and, in particular, the influence of HCV on this process within the liver.

Reference: Hepatology. 2015;61(3):843-56
Abstract

Sofosbuvir with peginterferon-ribavirin for 12 weeks in previously treated patients with hepatitis C genotype 2 or 3 and cirrhosis

Authors: Lawitz E et al.

Summary: This study examined the efficacy, safety and tolerability of sofosbuvir plus pegylated interferon and ribavirin (SOF+Peg-IFN+RBV) administered for 12 weeks to treatment-experienced patients with HCV genotypes 2 and 3, with and without cirrhosis. Forty-seven such patients aged ≥18 years were recruited and received study treatment. Twenty-three (49%) patients had HCV genotype 2 and 24 (51%) had genotype 3. Overall, 85% of patients achieved a sustained virological response (SVR) at 12 weeks after cessation of study treatment (SVR12). Rates of SVR12 were higher in patients with genotype 2 than in those with genotype 3 (96% and 83%, respectively). Rates of SVR12 were similar in patients with and without cirrhosis: for genotype 2, 93% of patients with cirrhosis and 100% of patients without cirrhosis achieved SVR12; corresponding SVR12 rates for genotype 3 were 83%, in patients both with and without cirrhosis. One patient discontinued study treatment because of an adverse event and four patients experienced serious adverse events. Influenza-like illness, fatigue, anaemia, and neutropenia were the most commonly recorded adverse events.

Comment: Previous studies have demonstrated that sofosbuvir, a first-in-class nucleotide analogue HCV NS5B polymerase inhibitor, in combination with PEG-IFN and ribavirin for 12 weeks is highly efficacious in non-cirrhotic, treatment-naïve patients with genotype 2 or genotype 3 HCV infection. This phase 2 analysis is the first to determine efficacy in PEG-IFN/ribavirin treatment-experienced genotype 2 or genotype 3 HCV patients, with or without cirrhosis. Overall, high rates of SVR were demonstrated, especially in genotype 2 patients (SVR12 96% compared to SVR12 83% in genotype 3 patients). Importantly, SVR12 rates were similar for both genotypes in patients with and without cirrhosis. Consequently, sofosbuvir/PEG-IFN/ribavirin should be seen as a worthwhile therapeutic option in genotype 2 and genotype 3 HCV patients who are able to tolerate 12 weeks of PEG-IFN treatment, especially those who have progressed to cirrhosis.

Reference: Hepatology. 2015;61(3):769-75
Abstract

Effect of sofosbuvir and ribavirin treatment on peripheral and hepatic lipid metabolism in chronic hepatitis C virus, genotype 1-infected patients

Authors: Meissner EG et al.

Summary: This investigation examined changes in serum lipid profiles and intrahepatic expression of lipid-related genes during IFN-free treatment (SOF+RBV) for genotype 1 chronic HCV infection and it explored associations with treatment outcome. On-treatment measurements of serum lipids revealed increases in serum LDL cholesterol and particle size early in therapy, whereas triglyceride (TG) levels and very-low-density lipoprotein particle size decreased, irrespective of treatment outcome. Whereas LDL increased in patients regardless of treatment outcome, average LDL concentration was lower at baseline and post-treatment in patients who relapsed. Paired pre- and end-of-treatment (EOT) liver biopsies from 8 patients (7 with SVR; 1 was a relapser) revealed altered expression of genes associated with lipid transport, assembly, and signalling. In unpaired EOT liver biopsies from 25 patients (17 with SVR; 8 were relapsers), intrahepatic expression of fatty acid metabolism and lipid transport genes was lower in those patients who relapsed.

Comment: There exists a close inter-relationship between HCV infection and lipid metabolism. HCV influences the expression of lipid-related genes, while lipids influence viral biology. This study investigates the impact of sofosbuvir and ribavirin therapy on both lipid metabolism and the hepatic expression of genes involved in lipid homeostasis in patients with genotype 1 HCV infection. The results support the concept that HCV alters lipid metabolism, resulting in improved viral fitness, reduced likelihood of viral eradication and, ultimately, disease progression. Effective antiviral therapy with sofosbuvir and ribavirin restores normal lipid metabolism. The findings further raise the possibility that consideration of lipid metabolites in peripheral blood may be useful to predict treatment response to direct-acting antiviral therapy.

Reference: Hepatology. 2015;61(3):790-801
Abstract

Hepatitis B surface antigen genetic elements critical for immune escape correlate with hepatitis B virus reactivation upon immunosuppression

Authors: Salpini R et al.

Summary: Hepatitis B surface antigen (HBsAg) genetic features underlying HBV reactivation during immunosuppression were examined in 29 patients developing HBV reactivation and 64 with chronic HBV infection (controls). Prior to HBV reactivation, 51.7% of patients were isolated hepatitis B core antibody (anti-HBc)-positive, 31.0% inactive carriers, 6.9% anti-HBc/anti-HBs (hepatitis B surface antibody)-positive, 6.9% isolated anti-HBs-positive, and 3.4% had an overt HBV infection. Among the HBV reactivation group, 51.7% received rituximab, 34.5% different chemotherapeutics, and 13.8% corticosteroids only for inflammatory diseases. In total, 75.9% of HBV-reactivated patients (vs 3.1% of controls; $p<0.001$) carried HBsAg mutations localised in immune-active HBsAg regions. Eight of the 13 HBsAg mutations (M103I-L109I-T118K-P120A-Y134H-S143L-D144E-S171F) found in these patients reside in a major hydrophilic loop (target of neutralising antibodies [Abs]); some of them are already known to hamper HBsAg recognition by humoral response. The remaining 5 (C48G-V96A-L175S-G185E-V190A) are localised in class I/II-restricted T-cell epitopes, suggesting a role in HBV escape from T cell-mediated responses. Ultra-deep sequencing revealed that these mutations occurred in HBV-reactivated patients with a median intrapatient prevalence of 73.3% and in just 4.6% of controls carrying such mutations ($p<0.001$). Additional N-linked glycosylation sites within the major hydrophilic loop were found in 24.1% of HBV-reactivated patients (vs none of the chronic patients; $p<0.001$); 5 of 7 patients carrying these sites remained HBsAg-negative despite HBV reactivation.

Comment: The potential for reactivation of HBV in patients with apparently resolved primary HBV infection is due to the persistence within hepatocytes of covalently closed circular DNA, which serves as a template for the production of RNAs necessary for viral replication. This study is one of the first analyses from outside of Asia to address the genetic complexity of HBsAg sequences in patients with HBV reactivation due to immunosuppression. The findings show that HBV reactivation in this circumstance correlates with the presence of HBsAg mutations in immune-active sites and additional N-linked glycosylation sites that together promote an increased likelihood of immune escape. Otherwise, the study is yet another timely reminder of the importance of antiviral prophylaxis for at-risk patients.

Reference: Hepatology. 2015;61(3):823-33
Abstract

Estimation of hepatitis C virus infections resulting from vertical transmission in Egypt

Authors: Benova L et al.

Summary: These researchers constructed a mathematical model based on maternal HCV antibody and viraemia to estimate the absolute number of new HCV infections resulting from vertical transmission in Egypt, both nationally and for 6 subnational areas. Upon applying two vertical transmission risk estimates to the 2008 Egyptian birth cohort, the study researchers estimated that between 3080 and 5167 HCV infections resulted from vertical transmission among children born in 2008. HCV vertical transmission may account for half of incident cases in the <5-year age group. Disproportionately higher proportions of vertical infections were estimated in Lower Rural and Upper Rural subnational areas. The researchers explain that this geographical clustering was due to higher-area-level HCV prevalence among women and higher fertility rates.

Comment: Egypt has the highest prevalence of HCV of any country in the world, linked at least in part to a mass parenteral anti-schistosomal therapy campaign spanning the 1950s to 1980s. Using a conceptual framework of HCV vertical transmission based on a mathematical model, this analysis is the first published report to estimate the importance of vertical transmission to the incidence of HCV in Egypt. Overall, vertical transmission was estimated to represent around 5% of all incident HCV cases in Egypt, but could account for up to two-thirds of HCV incidence in children up to 5 years of age. Further research to better understand factors responsible for mother-to-infant transmission of HCV and to assess potential interventions that may reduce the risk of vertically-acquired infection is clearly warranted.

Reference: Hepatology. 2015;61(3):834-42
Abstract

Long-term efficacy of a hepatitis E vaccine

Authors: Zhang J et al.

Summary: Data are reported from an extended follow-up to an initial efficacy study conducted in China, in which healthy adults (aged 16–65 years) were randomised to receive 3 doses of either a hepatitis E vaccine (vaccine group; n=56,302) or a hepatitis B vaccine (control group; n=56,302). The vaccines were administered at 0, 1, and 6 months, and the participants were followed for 19 months. Thereafter, follow-up assessments of efficacy, immunogenicity, and safety were continued for up to 4.5 years. During the entire study period, there were 7 confirmed cases of hepatitis E in the vaccine group (0.3 cases per 10,000 person-years) and 53 in the control group (2.1 cases per 10,000 person-years), yielding a vaccine efficacy of 86.8% (95% CI, 71 to 94). Among the 7 cases in the group that received the vaccine, only 3 subjects had received all 3 doses. Of the participants who were assessed for immunogenicity and were seronegative at baseline, 87% of those who received 3 doses of the hepatitis E vaccine maintained antibodies against hepatitis E virus (HEV) for at least 4.5 years; HEV antibody titres developed in 9% in the control group. The rate of adverse events was similar in the two groups.

Comment: The results of this study that enrolled over 110,000 healthy participants in China suggest that HEV infection can largely be prevented by vaccination. The vaccine employed was well tolerated and remained immunogenic at least 4.5 years after a three-dose schedule delivered over 6 months. Efficacy was demonstrated particularly against genotype 4, the most common HEV genotype in China. Additional studies will be required to establish the efficacy of the vaccine in parts of the world where other HEV genotypes predominate. Cost-effectiveness of vaccination as a prevention tool also remains to be determined, while additional safety and efficacy data will be necessary in persons with chronic liver disease, pregnant women and other vulnerable populations before HEV vaccination can be widely recommended.

Reference: N Engl J Med. 2015;372(10):914-22
Abstract

Interleukin-28 gene polymorphisms may contribute to HBsAg persistence and the development of HBeAg-negative chronic hepatitis B

Authors: Karatayli SC et al.

Summary: These researchers sought to determine whether several single nucleotide polymorphisms (SNPs) of the interleukin (*IL*)*28B* gene (rs12979860, rs1188122, rs8099917, rs8105790, rs12980275) are potentially associated with HBsAg persistence. The analysis also assessed the development of HBeAg-negative chronic HBV versus inactive HBsAg carrier state in patients with genotype D HBV, and conducted a comparison between patients with chronic hepatitis D virus (HDV) and patients with chronic HBV. The study included 3 main patient cohorts: Group 1 consisted of 482 patients with HBsAg persistence; 143 were inactive carriers, 94 had HBeAg-positive chronic HBV and 245 had anti-HBe-positive chronic HBV. Group 2 contained spontaneously recovered HBV patients who were anti-HBs and anti-HBc-positive. Group 3 consisted of 176 patients with chronic HDV with anti-delta and HDV-RNA positivity. A comparison between patients with HBsAg persistence and spontaneously recovered patients revealed significant differences between the groups for rs8105790 ($p<0.0001$) and rs12980275 ($p<0.02$). In the comparison between inactive HBsAg carriers and HBeAg-negative patients with chronic HBV, patients who had the CC/TC genotype for rs8105790 ($p<0.0001$) and AA genotype for 1188122 ($p<0.02$) were more likely to be inactive HBsAg carriers. When patients with chronic HDV were compared with recovered HBV patients, results reflected the comparison of HBV persistence versus recovered HBV, with similar significant differences in the same SNPs.

Comment: Polymorphisms of the *IL28B* gene have been shown to influence spontaneous and treatment-induced clearance of HCV. This analysis sought to investigate any possible link between *IL28B* gene polymorphisms and the outcome of HBV infection in a HBV genotype D-restricted cohort in Turkey. The study is the first to suggest, firstly, an association between *IL28B* polymorphisms and HBsAg persistence and, in particular, the development of an inactive phase of infection. Further analyses performed in other geographical locations and including other HBV genotypes will be required to determine the full impact of *IL28B* polymorphisms on the natural history of HBV infection.

Reference: Liver Int. 2015;35(3):846-53
Abstract

