Hepatitis Research Review™

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In this issue:

Abbreviations used in this issue:

ApoE = apolipoprotein E; **DAA** = direct-acting antiviral; **ESRD** = end-stage renal disease; **HBV** = hepatitis B virus; **HCV** = hepatitis C virus; **HDV** = hepatitis D virus; **HEV** = hepatitis E virus; **hNTCP** = human sodium taurocholate cotransporting polypeptide; **HR** = hazard ratio; **HSCs** = hepatic stellate cells; **IHC** = immunohistochemistry; **NK** = natural killer; **SVR12** = sustained virological response at 12 weeks after end of therapy; **TDF** = tenofovir disoproxil fumarate.

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Welcome to issue 47 of Hepatitis Research Review.

Amongst the papers in this issue is one from Korean researchers who report that a ginsenoside compound (G-Rg3) strongly inhibits hepatitis C virus (HCV) propagation. Moreover, G-Rg3 reversed mitochondrial damage caused by HCV infection when used in combination with direct-acting antiviral therapy. In another paper, oral antibiotics lowered the intrahepatic accumulation of pro-inflammatory $CD14^{+}CD206^{+}$ myeloid cells in advanced viral-related liver disease. This finding could point to a new strategy in the management of patients with viral-induced cirrhosis.

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

Kind Regards,

Professor Stephen Riordan

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Sodium taurocholate cotransporting polypeptide is the limiting host factor of hepatitis B virus infection in macaque and pig hepatocytes

Authors: Lempp FA et al.

Summary: The human sodium taurocholate cotransporting polypeptide (hNTCP) is identified as being a key receptor for hepatitis B virus (HBV) and hepatitis D virus (HDV) entry into human hepatocytes. Disappointingly, complementation of mouse hepatocytes with hNTCP confers susceptibility to HDV but not HBV infection, which has encouraged the search for additional HBV-specific factors. These researchers examined how hNTCP overexpression promotes HBV and HDV infection in primary hepatocytes from mice, rats, dogs, pigs, rhesus macaques, and cynomolgus macaques. Hepatocytes were transduced with adeno-associated viral vectors encoding hNTCP and subsequently infected with HBV. Cells were analysed for Myrcludex B binding, taurocholate uptake, HBV covalently closed circular DNA formation, and expression of all HBV markers. Sodium taurocholate cotransporting polypeptide (Ntcp) from the respective species was cloned and analysed for HBV and HDV receptor activity in the Huh7 human hepatoma cell line. hNTCP-overexpressing hepatocytes from mice, rats and dogs were refractory to HBV infection but permitted HDV infection, whereas hepatocytes from macaques (rhesus and cynomolgus) and pigs became fully susceptible to HBV upon hNTCP expression with efficiencies comparable to human hepatocytes.

Comment: The hNTCP is a key receptor for entry of HBV and HDV into human hepatocytes. This study investigated the possible species-specific function of NTCP by studying the potential impact of over-expression of hNTCP on HBV and HBV infection of primary hepatocytes from various animal species. An important finding is that hepatocytes of macaques and pigs become fully susceptible to both HBV and HDV infection when transcomplemented with hNTCP. This observation raises the possibility of developing immunocompetent animal models to facilitate the preclinical assessment of novel immunotherapeutic approaches, including proof-of-concept studies.

Reference: Hepatology. 2017;66(3):703-16

Abstract

Elbasvir/grazoprevir for patients with hepatitis C virus infection and inherited blood disorders: a phase III study

Authors: Hézode C et al.

Summary: The multinational C-EDGE IBLD phase 3 study assessed the safety and efficacy of elbasvir and grazoprevir combination therapy in 159 adults with HCV infection and inherited blood disorders (sickle cell anaemia, thalassaemia, or haemophilia A/B or von Willebrand disease). The study randomised 107 patients to immediate treatment with the oral, once-daily, fixed-dose combination of elbasvir and grazoprevir 50 mg/100 mg for 12 weeks (ITG) or to a deferred treatment group (DTG; placebo followed by active treatment; n=52). In the ITG arm, 100 patients (93.5%) achieved SVR12, 6 relapsed, and 1 was lost to follow-up. SVR12 was achieved in 94.7% (18 of 19), 97.6% (40 of 41), and 89.4% (42 of 47) of patients with sickle cell disease, β-thalassaemia, and haemophilia A/B or von Willebrand disease, respectively. Serious adverse events were reported by 2.8% of patients in the ITG arm and by 11.5% of those in the DTG arm. Haemoglobin levels and international normalised ratio values were similar between the elbasvir/grazoprevir and placebo treatment cohorts. Among patients with haemoglobinopathies (sickle cell disease and β-thalassaemia), the on-treatment change in mean haemoglobin levels was similar between those receiving elbasvir/grazoprevir and those receiving placebo.

Comment: Direct-acting antivirals (DAAs) for chronic HCV infection have been little studied in patients with inherited blood disorders, such as haemoglobinopathies and haemolytic anaemia. This randomised, phase III study aimed to assess efficacy and safety of combination therapy for 12 weeks with elbasvir, a non-structural (NS) 5A HCV inhibitor and grazoprevir, a NS3/4A HCV inhibitor, in genotype 1, 4 or 6 HCV-infected patients with sickle cell disease, thalassaemia, haemophilia or von Willebrand disease, including those with and without compensated cirrhosis. High efficacy was demonstrated, with an overall sustained virological response rate 12 weeks after treatment completion of 93.5%. The safety profile was similar to that of a placebo. The data add to existing literature pointing to the effectiveness and tolerability of elbasvir and grazoprevir for the treatment of chronic HCV infection.

Reference: Hepatology. 2017;66(3):736-45
Abstract

Hepatitis C virus-induced CCL5 secretion from macrophages activates hepatic stellate cells

Authors: Sasaki R et al.

Summary: This investigation sought to clarify the mechanism of liver disease progression during HCV infection, by examining crosstalk between macrophages and hepatic stellate cells (HSCs) following HCV infection. Conditioned medium from HCV-exposed human macrophages was used to incubate primary human HSCs and immortalised HSCs (LX2 cells). Expression of inflammasome and fibrosis-related genes in these cells was examined, with increased expression of inflammatory (NLR family pyrin domain containing 3, interleukins 1β and 6, and cysteine-cysteine chemokine ligand 5 [CCL5]) and profibrogenic (transforming growth factor β1, collagen type 4 α1, matrix metalloproteinase 2, and α-smooth muscle actin) markers. Inflammasome and fibrosis markers in HSCs appeared to be activated by CCL5 secreted from HCV-exposed macrophages, while anti-CCL5 neutralising antibody downregulated activation.

Comment: Macrophages, including Kupffer cells, produce proinflammatory cytokines when exposed to HCV. These cytokines in turn activate HSCs and promote hepatic fibrosis. This study employed primary HSCs and an immortalised HSC line (LX2 cells), incubated with conditioned medium derived from HCV-exposed macrophages, to further investigate molecular cross-talk between macrophages and HSCs in the context of HCV infection. The findings indicate that HCV induces the secretion of CCL5 by macrophages, with CCL5 in turn activating both inflammatory molecules and markers of fibrosis in HSCs. The observations are important, in that an improved understanding of the detailed mechanisms involved in HSC activation towards a profibrotic phenotype and how this can be inhibited will be crucial to the development of future treatment strategies to limit and reverse HCV-mediated hepatic fibrosis.

Reference: Hepatology. 2017;66(3):746-57
Abstract

Ginsenoside Rg3 restores hepatitis C virus-induced aberrant mitochondrial dynamics and inhibits virus propagation

Authors: Kim SJ et al.

Summary: This *in vitro* investigation into the anti-HCV activity of several ginsenoside compounds identified that ginsenoside Rg3 (G-Rg3) is a strong inhibitor of HCV propagation. G-Rg3 treatment of HCV-infected cells increased HCV core protein-mediated reduction in the expression level of cytosolic p21, required for increasing cyclin-dependent kinase 1 activity, which catalyses Ser616 phosphorylation of dynamin-related protein 1. G-Rg3 also rescued HCV-infected cells from mitophagy.

Comment: Ginseng has long been used as a traditional herbal remedy in Asian medicine, with the ginsenoside compounds in ginseng exerting a range of immunological and pharmacologic effects. This study demonstrates that ginsenoside Rg3 (G-Rg3) has antiviral activity against HCV infection. In particular, G-Rg3 opposes HCV-mediated abnormal mitochondrial fission and mitophagy, phenomena that promote persistent HCV infection. G-Rg3 was found to reverse mitochondrial damage caused by HCV infection when used in combination with DAA therapy, supporting the rationale for its use as an adjunctive treatment in this setting. Nonetheless, on a cautionary note, G-Rg3 can be readily metabolised to the potentially toxic metabolite, G-Rh2, by various bacterial populations in the gastrointestinal tract, raising the possibility that tolerability and efficacy may vary across individual patients depending upon the particular composition of their gut microbiome.

Reference: Hepatology. 2017;66(3):758-71
Abstract

Monotherapy with tenofovir disoproxil fumarate for multiple drug-resistant chronic hepatitis B: 3-year trial

Authors: Lim YS et al.

Summary: Outcomes are reported from 189 patients with HBV-resistance mutations to entecavir (ETV) and/or adefovir, who had been treated with either tenofovir disoproxil fumarate (TDF) alone or in combination with entecavir (TDF/ETV) for 48 weeks, and agreed to continue TDF monotherapy (TDF-TDF group) or to switch to TDF monotherapy (TDF/ETV-TDF group) for up to 144 weeks. 180 (93.8%) completed the 144-week study. The primary efficacy endpoint, serum HBV DNA <15 IU/mL at week 48, was achieved by similar proportions of patients in each treatment group (66.3% of the TDF-TDF group and 68.0% of the TDF/ETV-TDF group; p=0.80). At week 144, the proportion with HBV DNA <15 IU/mL was significantly increased from week 48 to 74.5% (p=0.03), with no significant between-group difference (p=0.46). Transient virological breakthrough occurred in 6 patients, which was due to poor drug adherence. At week 144, results of genotypic resistance analysis performed for 19 patients who had HBV DNA levels >60 IU/mL revealed that 6 had ≥1 detectable HBV resistance mutation, all of which were present at baseline. No cases of additional resistance mutations were reported during study treatment.

Comment: Persistent replication of HBV is an independent risk factor for disease progression to cirrhosis and hepatocellular carcinoma in patients with chronic HBV infection. The reduction in HBV DNA concentrations to very low or undetectable levels by the long-term use of nucleos(t)ide analogue therapy is associated with reduced risk of hepatocellular carcinoma development and mortality. Previous studies have demonstrated that monotherapy with TDF for 48 weeks is as efficacious as combination TDF plus entecavir therapy in patients with documented resistance to entecavir and adefovir dipivoxil. This trial aimed to establish the safety and efficacy of prolonged TDF monotherapy for up to 144 weeks in this circumstance as an extension of previous analyses. The findings indicate that prolonged TDF monotherapy is well tolerated and gradually increases the rate of virological response, with no additional development of resistance but rather a marked reduction in detectable resistance mutations of HBV. Taken together, TDF monotherapy appears an appropriate long-term treatment option for patients with entecavir- and adefovir dipivoxil-resistant chronic HBV infection.

Reference: Hepatology. 2017;66(3):772-83
Abstract

Hepatitis C viral load, genotype, and increased risk of developing end-stage renal disease: REVEAL-HCV study

Authors: Lai TS et al.

Summary: This Taiwanese community-based prospective cohort study sought to determine whether HCV RNA level and genotype are independent risk factors for developing end-stage renal disease (ESRD). The study enrolled 19,984 participants aged 30–65 years between 1991 and 1992. After a median 16.8-years of follow-up, 204 ESRD events had occurred during 319,474 person-years. The incidence rates of ESRD for nonchronically HCV-infected and chronically HCV-infected patients were 60.2 and 194.3 per 100,000 person-years, respectively. In a comparison of patients with and without chronic HCV infection, the multivariable-adjusted hazard ratio was 2.33 (95% CI, 1.40 to 3.89). Compared with patients who did not have chronic HCV infection, patients with low and those with high HCV RNA levels had a higher risk of developing ESRD (HR 2.11; 95% CI, 1.16 to 3.86, and HR 3.06; 95% CI, 1.23 to 7.58; $p_{trend} < 0.001$). This association persisted after taking pre-ESRD death as a competing event for ESRD. Patients with HCV genotype 1 had a higher risk of developing ESRD compared with patients who did not have chronic HCV infection (HR 3.60; 95% CI, 1.83 to 7.07).

Comment: An association between HCV infection and glomerulonephritis is well known. Understanding of the possible relationship between chronic HCV infection and ESRD is less well-established. This large-scale, community-based cohort study of nearly 20,000 participants performed in Taiwan addressed this issue. The findings suggest that chronic HCV infection is an independent risk factor for the development of ESRD, with an overall increased risk in the order of 2.2-fold. The magnitude of the increased risk was related to viral load, with those with HCV RNA levels below and above 167,000 IU/mL found to have 2.1-fold and 3.1-fold increased risks of ESRD, respectively, compared to those without chronic HCV infection. ESRD risk also appeared genotype-related, with those with chronic genotype 1 HCV infection particularly likely to have developed ESRD during 15 years of follow-up. Mechanisms underlying this apparent association between HCV genotype and risk of ESRD remain to be determined.

Reference: Hepatology. 2017;66(3):784-93
Abstract

HLA-Bw4 80(T) and multiple HLA-BW4 copies combined with KIR3DL1 associate with spontaneous clearance of HCV infection in people who inject drugs

Authors: Thöns C et al.

Summary: Natural killer (NK) cell function is regulated by inhibitory and activating receptors including killer-cell immunoglobulin-like receptors (KIRs). These researchers assessed the impact of different genetic KIR/KIR-ligand combinations on the outcome of HCV infection in people who inject drugs (PWID). HLA class I alleles with the Bw4 80(T) motif or multiple copies of HLA-Bw4 alleles in combination with its receptor KIR3DL1 were associated with a protective state against chronic HCV infection in a cohort of 266 PWID from Germany and confirmed in a North American cohort of 342 anti-HCV-positive PWID. In multivariate logistic regression analysis, *KIR3DL1*/HLA-Bw4 80(T) was associated with spontaneous clearance of HCV infection in PWID, which was confirmed in the PWID cohort from North America. Compared with PWID with detectable HCV RNA, the frequency of individuals with multiple HLA-Bw4 alleles was significantly higher in anti-HCV positive PWID with resolved HCV infection (29.7% vs 15.2%; p=0.0229) and in anti-HCV seronegative PWID (39.2%; p=0.0006). KIR3DL1$^+$ NK cells from HLA-Bw4 80(T)-positive PWID showed superior functionality compared to HLA-Bw4 80(I)-positive PWID. This differential functionality of KIR3DL1$^+$ NK cells in the presence of HLA-Bw4 80(T) and Bw4 80(I) was not observed in healthy donors.

Comment: The natural killer (NK) cell arm of the innate immune system is regulated by a complex network of genetically-determined receptor-ligand pairs. In this study, the authors identified a particular set of NK receptor and ligand genes that confer increased functionality of NK cells and improved outcomes following HCV exposure in a high-risk group injecting parenteral drugs. In particular, analysis of a cohort in Germany demonstrated that HLA class I alleles with the Bw4 80(T) motif or multiple copies of HLA-Bw4 alleles in combination with its receptor, KIR3DL1, are associated with protection against chronic HCV infection in such subjects. The findings were confirmed in a second cohort recruited in North America. The results add to existing literature that points to an important role of NK cells in determining outcome of HCV infection and emphasise, in particular, the beneficial effect of the interaction between the KIR3DL1 receptor and its ligand, HLA-Bw4.

Reference: J Hepatol. 2017;67(3):462-70
Abstract

Visualization of hepatitis E virus RNA and proteins in the human liver

Authors: Lenggenhager D et al.

Summary: These researchers describe their method for visualising hepatitis E virus (HEV) infection in liver tissue sections. They evaluated a panel of 12 different antibodies against HEV open reading frame (ORF) 1-3 proteins stained for immunohistochemistry (IHC) and two probes for *in situ* hybridization (ISH) in formalin-fixed, paraffin-embedded (FFPE) HuH7 cells transfected with HEV ORF1-3 expression vectors. They applied IHC (and partly ISH) to Hep293TT cells replicating infectious HEV and liver specimens from 20 patients with HEV infection and 134 healthy controls. ORF1-3 proteins were all detectable in transfected, HEV protein-expressing cells, but only ORF2 and 3 proteins were traceable in HEV-infected cells. ORF2-encoded capsid protein was reliably detected in liver specimens from patients with HEV infection. IHC for ORF2 protein revealed a patchy expression in individual or grouped hepatocytes, generally stronger in chronic versus acute HEV infection. ORF2 protein was also detected in the nucleus of HEV-infected cells. Positivity for ORF2 protein in defined areas correlated with HEV RNA detection by ISH. The study researchers state that IHC was specific and as sensitive as polymerase chain reaction (PCR) for HEV RNA.

Comment: Although mostly asymptomatic, HEV is the leading cause of acute viral hepatitis worldwide. Diagnosis is made by the detection of anti-HEV antibodies in peripheral blood and HEV RNA in blood or stool, the latter using PCR methodology. Serological testing is often not sufficient, given the high underlying rates of anti-HEV-IgG seropositivity of up to 20%, even in developed countries, and suboptimal sensitivity and specificity of anti-HEV-IgM assays. Furthermore, PCR testing for HEV can be problematic as assays show variable sensitivity and lack standardisation, while HEV viraemia and faecal excretion of HEV occur during only a limited time period. Here, the authors developed an IHC staining technique to detect HEV in liver tissue as a potentially helpful addition to currently available HEV diagnostics, particularly in hard-to diagnose settings in which liver tissue becomes available for testing. Nonetheless, sensitivity was not ideal. In particular, compared to an established PCR method for HEV viraemia, hepatic IHC analysis was found to have 76% sensitivity for HEV infection at high levels of viraemia but only a problematic 6% sensitivity at low levels of viraemia, such that negative testing of liver tissue by this methodology cannot reliably be taken to exclude HEV infection.

Reference: J Hepatol. 2017;67(3):471-9
Abstract

Intrahepatic CD206⁺ macrophages contribute to inflammation in advanced viral-related liver disease

Authors: Tan-Garcia A et al.

Summary: This paper describes how intrahepatic CD14⁺ myeloid cells contribute to chronic liver inflammation in patients with viral-related liver disease. The researchers performed detailed phenotypic, molecular and functional analyses on intrahepatic CD14⁺ myeloid cells from 19 healthy donors and 15 patients with viral-related liver cirrhosis (HBV, HBV/HDV or HCV). In unsupervised analysis of multi-parametric data, liver disease was associated with the intrahepatic expansion of activated myeloid cells mainly consisting of pro-inflammatory CD14⁺HLA-DR^{hi}CD206⁺ cells, which spontaneously produced tumour necrosis factor-alpha (TNF-α) and granulocyte-macrophage colony-stimulating factor (GM-CSF). These cells only showed heightened pro-inflammatory responses to bacterial toll-like receptor (TLR) agonists and were more refractory to endotoxin-induced tolerance. A liver-specific enrichment of CD14⁺HLA-DR^{hi}CD206⁺ cells was also detected in a humanised mouse model of liver inflammation. Oral antibiotics lowered the intrahepatic accumulation of pro-inflammatory CD14⁺CD206⁺ myeloid cells.

Comment: Mechanisms by which chronic liver inflammation is maintained in patients with viral-mediated cirrhosis are not well understood. Here, the authors investigated the contribution of intrahepatic CD14⁺ myeloid cells to this phenomenon. They found that livers of such patients were infiltrated with activated myeloid cells, in particular CD14⁺HLA-DR^{hi}CD206⁺ cells, that spontaneously produced pro-inflammatory mediators and showed both increased responses to stimulation by bacterial products and a higher resistance to lipopolysaccharide tolerance compared to CD14⁺CD206⁻ myeloid cells. Treatment with oral antibiotics normalised intrahepatic CD14⁺HLA-DRC^{hi}D206⁺ cell numbers in an HBV-infected humanised mouse model. Taken together, the findings suggest that liver inflammation in advanced, viral-related chronic liver disease can be sustained by a pathological interaction between intrahepatic CD14⁺HLA-DR^{hi}CD206⁺ myeloid cells and bacterial products derived from the intestine and that strategies to interrupt this harmful interaction should be given additional focus in the management of patients with viral-induced cirrhosis.

Reference: J Hepatol. 2017;67(3):490-500
Abstract

Maturation of secreted HCV particles by incorporation of secreted ApoE protects from antibodies by enhancing infectivity

Authors: Bankwitz D et al.

Summary/Comment: HCV replication is influenced by host factors and cellular pathways involved in lipid metabolism and remodelling, such as apolipoprotein E (ApoE), which facilitates virus attachment. Direct interactions between ApoE and HCV proteins, including non-structural protein 5A and envelope proteins 1 and 2, have been well described, while lack of ApoE expression is known to prevent assembly of infectious viral progeny after the envelopment of capsids. This study investigated whether ApoE released from non-infected cells interacts with and modulates secreted HCV particles. The findings demonstrate that physiological levels of ApoE released from hepatocytes enhance HCV particle infectivity across all HCV genotypes by incorporating into virus particles, both enhancing interactions with cellular heparin sulfate proteoglycans and facilitating evasion from neutralising antibodies. Strategies to interfere with this ApoE/HCV interaction may be useful both to limit HCV infectivity and increase the efficacy of any future prophylactic HCV vaccines to limit the global impact of HCV infection.

Reference: J Hepatol. 2017;67(3):480-9
Abstract

Independent commentary by Professor Stephen Riordan, Senior Staff Specialist, Gastrointestinal and Liver Unit, Prince of Wales Hospital and Conjoint Professor of Medicine, University of New South Wales, Sydney.

