Welcome to issue 28 of Hepatitis Research Review.

One of the papers in this issue describes the detection of hepatitis E virus (HEV) RNA and antigen (HEV-Ag) in urine of patients with chronic or acute HEV infection and HEV-infected monkeys. There was evidence of kidney injury in the monkeys. Interestingly, one monkey became infected with HEV after inoculation with urine from another infected monkey. The study researchers suggest that HEV-Ag detection in urine may assist in the diagnosis of ongoing HEV infection.

In another paper, findings from an open-label study show that combination treatment with tenofovir disoproxil fumarate and peginterferon α-2a achieved hepatitis B surface antigen (HBsAg) loss in almost 1 out of 10 patients with chronic hepatitis B virus infection; rates of HBsAg loss were higher than those observed with either monotherapy.

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

Professor Stephen Riordan
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A targeted functional RNA interference screen uncovers glypican 5 as an entry factor for hepatitis B and D viruses

Authors: Perin PM et al.

Summary: These researchers sought to better understand the process of the viral cell entry process in hepatitis B and D infectivity. They constructed a high-throughput infectious cell culture model that used single clones of HuH7 cells stably infected with human sodium/taururonate co-transporting polypeptide (NTCP). Of 120 clones, one showed high susceptibility to hepatitis D virus (HDV) infection. The researchers detail how they used a targeted RNA interference entry screen to identify glypican 5 (GPC5) as a common host cell entry factor for hepatitis B and D viruses.

Comment: Mechanisms of entry of hepatitis B virus (HBV) into hepatocytes are incompletely understood. This study investigated the possible role of the heparin sulfate proteoglycans (HSPG) gene family, including agrin, syndecan 1-4 and glypican 1-6, in this process. Using clones of HepG2 and HuH7 human hepatoma cell lines and primary human hepatocytes, the silencing of the 12 HSPG genes by means of small interfering RNA (siRNA) pools and blocking experiments using specific HSPG antibodies and soluble forms, the authors present compelling evidence that GPC5 is a cell surface receptor for both HBV and hepatitis D virus (HDV). Since siRNAs only incompletely abolished GPC5 expression, it remains unclear, however, both if GPC5 is the only HSPG capable of acting as an HBV/HDV receptor and whether GPC5 is necessarily required for HBV/HDV infectivity.


Abstract

Flunarizine prevents hepatitis C virus membrane fusion in a genotype-dependent manner by targeting the potential fusion peptide within E1

Authors: Perin PM et al.

Summary: The study evidence in this paper demonstrates a potential therapeutic strategy for hepatitis C virus (HCV) infection. The study researchers screened a compound library including licensed drugs. In a series of experiments, flunarizine (a clinically approved anti-migraine therapy) inhibited HCV cell entry in vitro and in vivo in a genotype-dependent fashion. An analysis of mosaic viruses between susceptible and resistant strains revealed that E1 and E2 glycoproteins confer susceptibility to flunarizine. Further analyses demonstrated that flunarizine specifically prevents membrane fusion. Related phenothiazines (fluphenazine and trifluoperazine) and pimozide had similar capacity to flunarizine in inhibiting HCV infection and also preferentially targeted HCV genotype 2 viruses. Interestingly, the phenothiazines and related flunarizine-resistant HCV carried mutations within the alleged fusion peptide and displayed cross-resistance to these compounds, indicating that they are likely to share the inhibitory mechanism of flunarizine.

Comment: The entry of HCV into hepatocytes is a complex process involving multiple host entry factors. HCV E2 and E1 are envelope glycoproteins required for cellular attachment and membrane fusion, respectively. This study identifies flunarizine, a calcium channel antagonist with potential use in the treatment of vascular disorders, as an inhibitor of the fusion of genotype 2 HCV and host cell membranes during viral entry. The findings add membrane fusion to the list of potential targets for anti-HCV therapy, at least with regard to genotype 2 HCV infection, and may facilitate the development of additional, broader spectrum inhibitors of HCV entry, operative through inhibition of viral-host membrane fusion pathways.


Abstract
Interferon lambda 4 genotypes and resistance-associated variants in patients infected with hepatitis C virus genotypes 1 and 3

Authors: Peiffer K-H et al.

Summary/Comment: The single-nucleotide polymorphism (SNP) at rs12979860, formerly known as IL28B, resides within the interferon lambda 4 (IFNL4) gene and is known to favourably influence response to pegylated interferon (PEG IFN)-ribavirin treatment for chronic HCV infection. This study addressed the possible impact of IFNL4 status on naturally occurring HCV resistance-associated variants (RAVs) in a large European cohort and how any associations between IFNL4 and RAVs may impact response to direct-acting antiviral therapy. The findings are important in demonstrating that the non-structural 5A (NS5A) RAVs, Y939H, occurs not infrequently in genotype 1b-infected patients (14%) and is significantly associated with the “favourable” IFNL4 status, observations that may explain a lack of correlation or an inverse correlation between an apparently favourable IFNL4 genotype and response to NS5A-based direct-acting antiviral therapy in patients with genotype 1b HCV infection.


Abstract

Indoleamine-2,3-dioxygenase as an effector and an indicator of protective immune responses in patients with acute hepatitis B

Authors: Yoshio S et al.

Summary: These researchers explored the role of the enzyme indoleamine-2,3-dioxygenase (IDO) in HBV clearance in an infected cohort of patients. Acute hepatitis patients (n=25; chronic hepatitis, n=14; hepatic flare, n=14). IDO activity was also assessed in 14 healthy volunteers. The researchers used a culture model consisting of human natural killer cells, plasmacytoid dendritic cells, and HBV-transfected HuH7 cells. Acute hepatitis was associated with a robust activation of IDO and an inverse correlation of alanine aminotransferase at the peak, whereas this did not occur with hepatic flare. In acute hepatitis patients who eventually cleared HBV, IDO activity, chemokine (C-X-C motif) ligand 9 (CXCL9), CXCL10 and CXCL11 increased at the peak of alanine aminotransferase, whereas in patients with hepatic flare, IDO activity remained at lower levels, regardless of the surge of CXCL9, CXCL10, and CXCL11 at the alanine aminotransferase peak. IDO activity and HBV suppression in HuH7 cells was enhanced by co-existing natural killer cells and plasmacytoid dendritic cells producing interferon-γ and interferon-α. This suppressive effect of IDO on HBV was abolished in IDO-knockout cells and recovered after IDO was reintroduced.

Comment: Indoleamine-2,3-dioxygenase (IDO) is an enzyme, inducible by inflammatory mediators including interferon-gamma (IFN-γ) and interferon-alpha (IFN-α), that catalyses tryptophan to kynurenine. Reported effects include promotion of immune tolerance via T cell inhibition, while suppression of HBV replication in transfected HepG2 cells has also been documented. The role of IDO in patients with acute HBV infection is yet to be determined. This study included both a longitudinal clinical assessment and an in vitro culture model comprising HBV-positive HuH7 cells, natural killer (NK) cells and plasmacytoid dendritic cells (pDCs) and found that IDO activation in the early phase of acute HBV infection is important for viral clearance, likely via a non-cytotoxic effect. Synergistic production of IFN-γ and IFN-α by NK cells and pDCs enhanced IDO activity and its suppressive effect on HBV replication in vitro. The findings add to current understanding of mechanisms promoting viral clearance in acute HBV infection.


Abstract

Dysregulation of distal cholesterol biosynthesis in association with relapse and advanced disease in CHC genotype 2 and 3 treated with sofosbuvir and ribavirin

Authors: Younossi ZM et al.

Summary: These researchers assessed changes in the serum lipid and distal (post-squalene) cholesterol biosynthesis metabolite profile of 50 patients infected with HCV genotype (GT) 2 and 77 patients with HCV GT3, all of whom were treated with sofosbuvir and ribavirin. Serum samples obtained at baseline, after 12 weeks of treatment and again at 4 weeks post-treatment were analysed for apolipoproteins B and E (apoB/E), total cholesterol, HDL, LDL, and 11 post-squalene sterol metabolites. Half of the patients (50%) had cirrhosis and 42% experienced a virological relapse. At baseline, serum levels of lipids, apoB/E, 7-dehydrocholesterol, desmosterol and lathosterol were lower in the GT3 cohort than in the GT2 cohort (p<0.006). Baseline lathosterol was lower in relapsers with cirrhosis compared to cirrhotics who achieved a sustained virological response (SVR) (p=0.003). From baseline to treatment week 12, serum lipids, apoB/E, 7-dehydrocholesterol, desmosterol and lathosterol were lower in the GT3 cohort than in the GT2 cohort (p<0.006). Baseline lathosterol was lower in relapsers with cirrhosis compared to cirrhotics who achieved a sustained virological response (SVR) (p=0.003). From baseline to treatment week 12, serum lipids, apoB/E, and dihydropinoxosterol decreased with viral suppression (p<0.025). At 4 weeks post-treatment, cirrhotic SVR patients had substantially greater increases in apoB and total sterols compared to cirrhotic relapsers, regardless of HCV genotype. In analyses adjusted for genotype and gender, baseline lathosterol was independently associated with virological response (p=0.04).

Comment: Genotype 3 HCV infection is associated with a range of metabolic abnormalities that include dyslipidaemia. Pathogenic mechanisms underlying a genotype-specific disturbance of cholesterol metabolism are poorly understood. This study investigated this issue. The findings indicate that, compared with genotype 2 infection, genotype 3 HCV is associated with significant reductions in circulating levels of both apolipoproteins B and E and sterols involved in the distal cholesterol biosynthesis pathway (7-dehydrocholesterol, desmosterol and lathosterol), leading to relative hypocholesterolaemia. The latter changes are reversible with effective antiviral therapy. Cirrhotic patients with lower circulating lathosterol levels at baseline were found to have a reduced likelihood of response to antiviral therapy. Although responsible mechanisms remain to be defined, the findings are important in demonstrating a clear difference in the effects of genotypes 2 and 3 HCV with regard to lipid metabolism that may impact upon viral clearance.


Abstract

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Abstract
WHO CAN CHANGE WHAT’S POSSIBLE
BE THE
ONE
Albert Einstein used with permission of the HUJ/GreenLight.

Please review the Approved Product Information before prescribing. Full Product Information is available here.

PBS Information: This product is not listed on the PBS.

Minimum Product Information, HARVONI (ledipasvir/sofosbuvir) 90/400 mg tablets. INDICATIONS • Chronic hepatitis C (CHC) genotype 1 infection in adults. DOSAGE AND ADMINISTRATION • One tablet daily, orally. CONTRAINDICATIONS • Hypersensitivity, concurrent use with other medicinal products containing any of the same active components. PRECAUTIONS • Symptomatic bradycardia when coadministered with amiodarone. Use with potent P-gp Inducers. HCV/HBV co-infection. Patients with decompensated cirrhosis, patients with prior exposure to HCV direct-acting antivirals. Pregnancy (Category B1). DRUG INTERACTIONS • Acid reducing agents, antiarrhythmics (amiodarone), anticonvulsants, anticoagulants, antimycobacterials, antiretrovirals, simeprevir, St John’s wort, HMG-CoA reductase inhibitors. ADVERSE REACTIONS • Fatigue, headache, nausea, diarrhoea and insomnia, symptomatic bradycardia when coadministered with amiodarone. This is not a full list – for more details/complete list of adverse events refer to full Product Information. Date of preparation 21st July 2015.

FOR YOUR ADULT HCV GT1 PATIENTS¹
- 94–99% cure rate in GT1 patients with HARVONI¹–⁴a
- ≤1% of patients discontinued treatment with HARVONI due to adverse events¹
- HARVONI has a well-characterised DDI profile – please refer to PI before prescribing¹
- One tablet, once daily¹

¹Cure defined as SVR12 by the European Association for the Study of the Liver (EASL). DDI: drug-drug interaction.

References:

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Detection and assessment of infectivity of hepatitis E virus in urine

Authors: Meng Y et al.

Summary: Serum and urinary samples obtained from patients with chronic or acute hepatitis E virus (HEV) infection and HEV-infected monkeys were tested for the presence of HEV RNA and antigen (HEV-Ag) and its possible infectivity. HEV RNA and HEV-Ag were detected persistently in the urine of a patient with chronic HEV infection. HEV RNA was detected in the urine of 3 of the 8 (37.5%) acute patients, all of whom had detectable HEV-Ag in their urine. HEV RNA and HEV-Ag were also detectable in the urine of HEV-infected monkeys. The ratio of HEV-Ag to RNA in the urine of the infected monkeys was significantly higher than in their sera and faeces. While the parameters of routine urinanalysis remained within normal ranges in the clinical and animal populations, there were pathological changes and HEV-Ag was detected in the kidneys of the infected monkeys. Furthermore, 1 of 2 monkeys became infected with HEV after inoculation with urine from another infected monkey.

Comment: Understanding of the pathogenesis of HEV infection is relatively limited compared to that of hepatitis A virus. The present study has shown to be the predominant site of HEV replication, although extrahepatic replication is also recognised. This study extends current knowledge by confirming the presence of both HEV antigen and HEV RNA in urinary samples of patients with both acute and chronic HEV infection and also in an animal model. The findings demonstrate the infectivity of urinary HEV, thereby establishing for the first time a risk of HEV transmission through infected urine.

Reference: J Hepatol. 2016;64(1):37-43

Interferon-γ and tumor necrosis factor-α produced by T cells reduce the HBV persistence form, cccDNA, without cytolysis

Authors: Xia Y et al.

Summary: In this study, serum samples from patients with acute and chronic hepatitis B were analysed by a cytokine enzyme-linked immunosorbent assay. Hep52 HT-3 cells, HBV-infected HepaRG cells, and primary human hepatocytes were incubated with IFN-γ or tumour necrosis factor-α (TNF-α), or co-cultured with T cells. Levels of IFN-γ and TNF-α were increased in serum samples from patients with acute versus chronic hepatitis B and controls. In human hepatocytes with stably replicating HBV, as well as in HBV-infected primary human hepatocytes and HepaRG cells, IFN-γ and TNF-α each induced deamination of cccDNA and interfered with its stability; these cytokines had additive effects. Secretion of IFN-γ and TNF-α in an HBV-specific T cells inhibited HBV replication and reduced levels of HBV covalently closed circular DNA (cccDNA) in infected cells without the direct contact required for cytolysis. cccDNA decay was prevented by blocking IFN-γ and TNF-α. The finding suggests the need for continued investigation of cccDNA-deactivation pathways that are specific to HBV persistence.

Comment: This important study investigates mechanisms of elimination from hepatocytes of the nuclear form of the HBV genome, the covalently closed circular (ccc)DNA. The authors demonstrate that cccDNA levels in primary human hepatocytes infected with HBV are reduced via a non-cytotoxic effect of the pro-inflammatory cytokines, IFN-γ and TNF-α, secreted by HBV-specific T lymphocytes. In particular, these cytokines induce the expression of apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3A (APOBEC3A) and apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3B (APOBEC3B), leading to the deamination of cccDNA and its subsequent degradation by endonucleases. The findings suggest that it is the HBV capsid protein that recruits the APOBEC3 proteins to the cccDNA, although how this specifically occurs remains to be defined.


Sofosbuvir inhibits hepatitis E virus replication in vitro and results in an additive effect when combined with ribavirin

Authors: Dao Thi VL et al.

Summary/Comment: Although acute HEV infection is usually self-limited, persistent infection may develop in the immunocompromised, leading to chronic infection with risk of cirrhosis and liver failure. Approaches to management including reduction in pharmacological immunosuppression and treatment with PEG IFN or ribavirin are effective in promoting viral clearance in up to 75% of patients. In this in vitro analysis using subgenomic HEV replicons and cell culture, the authors found that sofosbuvir, now used for the treatment of chronic HCV infection, also inhibits HEV genotype 3, both as monotherapy and additively when used with ribavirin. The anti-HEV effect of sofosbuvir seems to be markedly less than its anti-HCV effect. Validation of these observations will require clinical studies, most appropriately in those chronic HEV patients who do not respond to current approaches to management.


Combination of tenofovir disoproxi fumurate and peginterferon α-2a increases loss of hepatitis B surface antigen in patients with chronic hepatitis B

Authors: Marcellin P et al.

Summary: This open-label trial enrolled 740 patients with chronic HBV infection and randomised them to receive tenofovir disoproxi fumurate (TDF), 300 mg once daily plus PEG IFN α-2a 180 µg weekly for 48 weeks (group A), TDF plus PEG IFN α-2a for 16 weeks followed by TDF alone for 32 weeks (group B), TDF monotherapy for 120 weeks (group C), or PEG IFN monotherapy for 48 weeks (group D). At week 72, loss of serum hepatitis B surface antigen (HBsAg) (the primary endpoint) occurred in 9.1% of subjects in group A, 2.8% of subjects in group B, none of the subjects in group C, and 2.8% of subjects in group D. HBsAg loss was significantly higher with 48 weeks of TDF plus PEG IFN α-2a than with TDF monotherapy (p<0.001) or PEG IFN α-2a alone (p<0.003). The proportions of subjects with HBsAg loss did not differ significantly between the monotherapy groups. HBsAg loss was achieved in both HBsAg-positive and HBsAg-negative patients and for all major viral genotypes. The incidence of common adverse events (including headache, alopecia, and pyrexia) and treatment discontinuation due to adverse events was similar among groups.

Comment: Whether combination treatment with a nucleos(t)ide analogue and interferon (IFN) is more effective than therapy with either agent alone in patients with chronic HBV infection is a topic of ongoing clinical importance, with conflicting results published to date. Here, an international consortium of investigators from 139 sites found in an open-label, randomised, controlled study of 740 non-cirrhotic, chronic HBV patients that rate of HBsAg loss was significantly greater with 48 weeks of combination TDF and PEG IFN α-2a than with either drug alone, although, overall, less than 10% of TDF + PEG IFN α-2a-treated patients lost HBsAg. Nonetheless, around one-third of a relatively small number of genotype A patients achieved this outcome with combination therapy. In addition to HBV genotype A, the presence of a marked hepatitis flare during the first 12 weeks of treatment, as reflected by an ALT level >10 times the upper limit of normal, was found to favour end-of-treatment response to combination antiviral therapy.


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