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Sofosbuvir monotherapy fails to achieve HEV RNA elimination in patients with chronic hepatitis E – The HepNet SofE pilot study

To the Editor:

Infection with HEV can lead to chronic hepatitis E in about 50% of immunosuppressed patients.[1] The management of chronic hepatitis E can be challenging and is a stepwise approach. Antiviral therapy is the second step if reduction of the immunosuppressive medication does not lead to viral clearance. However, options are limited and none of the drugs are approved for chronic hepatitis E. Interferon-alpha should not be considered in patients after lung, heart or kidney transplantation because it could induce graft rejection.[2] Ribavirin given for 3–6 months is usually the only option but side effects constrain its use, *e.g.* in anemic patients. Ribavirin is effective in approximately 80% of patients who can tolerate its use, meaning that 20% of treated patients remain viremic (EASL CPG).[3] Reasons for treatment failure could be selection of viral variants leading to enhanced viral replication.[4,5]

Thus, there is an unmet need for alternative safe and efficacious therapies. Recently, 2 studies demonstrated that the HCV polymerase inhibitor sofosbuvir inhibited HEV genotype 3 replication *in vitro*.[6,7] The combination of sofosbuvir and ribavirin resulted in an additive antiviral effect. However, in comparison to the strong antiviral effect of sofosbuvir on HCV *in vitro*, sofosbuvir had only a moderate effect on HEV. Despite this limited evidence, several patients who failed ribavirin or were ineligible for ribavirin therapy have been treated with sofosbuvir with or without ribavirin because of the imminent risk of developing liver decompensation. The results of the published case reports are conflicting.[8–13] Thus, we initiated the SofE study to investigate the antiviral efficacy and safety of sofosbuvir monotherapy in patients with chronic hepatitis E without the confounding effect of ribavirin.

This study was an investigator initiated, institutionally sponsored, prospective, multicenter, phase II pilot trial, conducted in 3 clinical sites in Germany. Patients with chronic HEV infection who were older than 18 years and who either failed to achieve clearance of infection after ribavirin therapy or had contraindications for ribavirin were eligible for inclusion. All patients had to be positive for HEV RNA at least 3 months before screening and HEV RNA positive at the time of screening.

After giving informed consent and screening of inclusion and exclusion criteria, patients received sofosbuvir 400 mg once daily for 24 weeks in an open labeled 1 arm trial design. The primary efficacy endpoint was defined as undetectable HEV RNA at week 24 (end of treatment). Secondary endpoints were durability of response 12 weeks after end of therapy and HEV RNA efficacy evaluations including HEV RNA changes from baseline during therapy and ALT normalization after 12 and 24 weeks of therapy and 12 weeks after end of therapy.

A detailed description of the study procedures, safety assessment, HEV sequence analyses and statistical analysis are listed in the supplementary information.

Twelve patients were screened and 10 patients entered the study (Fig. S1). However, 1 patient was excluded from the efficacy but not safety analysis because of a false positive HEV RNA at screening. Baseline characteristics of the 9 patients analyzed in the efficacy analyses are displayed in Table 1.

The individual HEV RNA kinetics during the entire study period are displayed in Fig. 1 and Fig. S2. Median HEV RNA levels declined from baseline to week 2 from 580,000 IU/ml to 45,000 IU/ml (1.1 log10 decline). However, HEV RNA increased in most patients between week 4 and end of treatment. The median HEV RNA was 100,000 IU/ml at week 12 (0.76 log10 decline) and 233,000 IU/ml at week 24 (0.40 log10 decline). At the end of the follow-up (12 weeks after end of therapy), the median HEV RNA level increased back to baseline levels (+0.11 log10 increase). The primary goal of the study, which was undetectable HEV RNA at the end of therapy, was not met in a single patient. Even a longer treatment duration may not achieve better results as the strongest antiviral effect was observed within the first 12 weeks of therapy. Nevertheless, 5 out of 9 patients treated with sofosbuvir achieved at least a 1.0 log10 reduction of HEV RNA, which may be considered as modest antiviral efficacy.

The results of this study are well in line with the *in vitro* data that showed a moderate antiviral effect that was far lower than the effect on HCV replication.[6,7] This indicates that the *in vitro* models used by the previous studies should be adequate to screen for further drug candidates, *e.g.* more specific HEV polymerase inhibitors. However, the study by Dao Thi *et al.* also showed an additive effect of sofosbuvir and ribavirin *in vitro*.[6] This additive effect may warrant a second study investigating sofosbuvir plus ribavirin in patients with chronic hepatitis E. Some of the positive case reports showed a signal that the combination may be more effective compared with a previous ribavirin monotherapy but others did not observe any antiviral effect.[8,11,13] However, most of the cases were already treated with ribavirin for several weeks or repeatedly. Accumulation of mutations associated with enhanced viral replication or drug resistance may have occurred[4,5] which could have limited the additive effect of ribavirin. Since ribavirin has been shown to induce HEV mutagenesis and selection of a variety of HEV variants, potential effects of distinct HEV mutations on the effect of sofosbuvir may be studied in more detail.

Interestingly, the antiviral effect of sofosbuvir might be influenced by the immunosuppressive co-medication. Patients treated with mammalian target of rapamycin (mTOR) inhibitors showed a weaker antiviral response, whereas patients treated with mycophenolate mofetil (MMF) showed stronger decreases of HEV RNA (Fig. S3). This observation would be in line with previous *in vitro* studies showing that mTOR inhibitors

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Table 1. Baseline characteristics and medical history (organ transplantation and immunosuppressive medication).

Patient	P1	P2	P3	P4	P5	P6	P7	P8	P9	Mean ± SD, or median (IQR)
Age (years)	37	61	42	47	30	60	36	59	24	44.0 ± 13.7
Sex	Female	Male	Female	Male	Female	Male	Male	Male	Male	
Duration of infection (months)	14	58	17	3	5	25	44	89	7	29.1 ± 29.13
HEV RNA (IU/ml)	80,000	350,000	580,000	4,800,000	4,600,000	160,000	474,000	805,000	1,000,000	$6E^5$ ($8E^4$–$5E^6$)
HEV genotype	3c	3c	3c	3 (na)	3c	3l	3c	3c	3c	
ALT (IU/L)	38	139	148	624	123	91	50	126	430	126 (38–624)
AST (IU/L)	33	88	81	349	72	88	41	93	238	88 (38–624)
Bilirubin (μmol/L)	5.0	6.0	8.6	5.1	6.8	10.3	6.0	10.6	19.0	6.8 (5–19)
Creatinine clearance (ml/min)	36.0	56.0	93.7	46.8	53.7	52.3	63.5	77.5	44.5	53.7 (36–93.7)
INR	1.0	0.9	1.0	0.9	1.0	1.1	1.0	1.1	1.2	1 (0.9–1.16)
Platelets (10^3/μl)	175	142	160	231	221	188	467	251	81	188 (81–467)
Fibroscan at screening LSM/IQR (kPa)	11.9/1.9	10.8/1.3	5.2/0.9	7.1/0.8	6.8/1.8	17.0/1.5	4.3/0.7	9.5/2.4	12.8/3.6	9.5 (4.3–17.0)
APRI score	0.5	1.2	1.4	3.0	0.9	0.9	0.2	0.7	5.9	
Cirrhosis*	No	Yes	Yes	No	No	Yes	No	No	Yes	
Duration of previous ribavirin therapy (days)	181	1,170#	180	Contra-indication[1]	Contra-indication[2]	567	450	1,380	126	
Mean dose of previous ribavirin (mg/d)	300	500	450	Contra-indication[1]	Contra-indication[2]	200	800	800	250	
Response to previous RBV therapy	RL	RL NR	RL	Contra-indication[1]	Contra-indication[2]	RL	NR	NR	NR	
Immune suppressive condition	Heart, kidney Tx	Kidney, kidney Tx	Stem cell Tx	Heart Tx	Kidney, pancreas Tx	Heart Tx	Multi–visceral	Kidney	CVID	
Immunosuprressive regimen										
Cortisone	x	x		x	x	x		x	x	
Evorolimus/sirolimus	x			x	x	x	x			
Tacrolimus	x	x		x	x		x	x		
Mycophenolate mofetil		x		x		x		x		

ALT, alanine aminotransferase; AST, aspartate aminotransferase; APRI, AST to platelet ratio index; CVID, common variable immunodeficiency; LSM, liver stiffness measurement; NR, non-response (breakthrough or never HEV RNA negative); RBV, ribavirin; RL, relapse (HEV RNA negative during and end of treatment); Tx, transplantation.
All non-responders had a >2-3 log decrease of HEV-RNA in the previous treatment, but the values increased again during treatment.
*Cirrhosis was defined either as fibroscan >14.5 kPa or APRI score >1.
[1]Contraindication: renal insufficiency.
[2]Contraindication: anemia, renal insufficiency.
#2 therapies: 1st therapy of 180 days with RL, 2nd therapy of 990 days with NR.

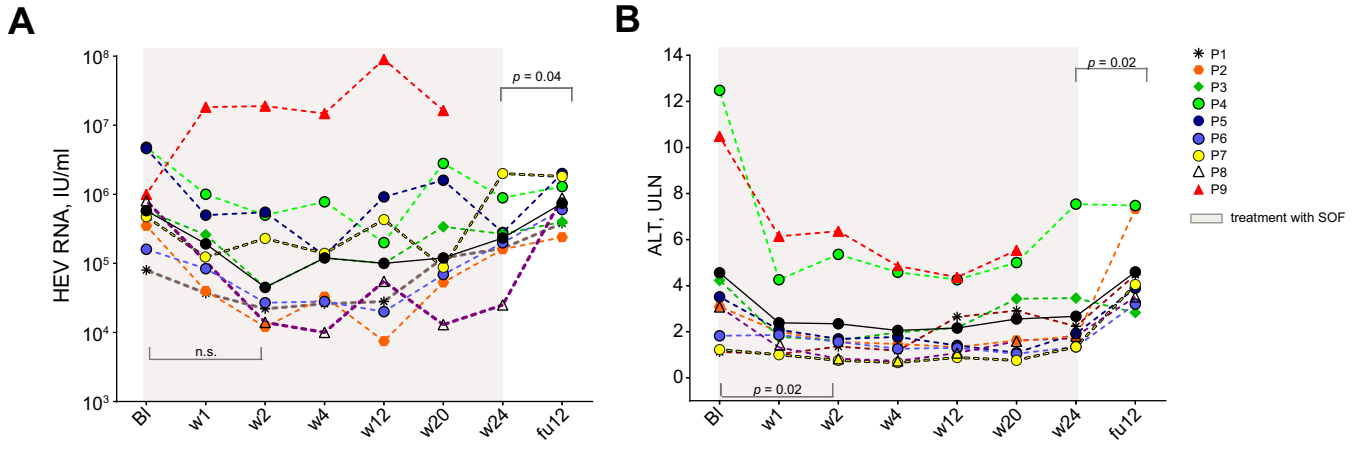

Fig. 1. Virological and biochemical response. (A) Individual and median HEV RNA (black line) from baseline until follow-up 12 weeks after the end of treatment. HEV RNA decreased between baseline and week 2 (not significant) and increased significantly after the end of treatment ($p = 0.04$ paired t test.). (B) Individual and mean ALT (black line) values depicted as ULN. ALT decreased significantly between baseline and week 2 ($p = 0.02$ paired t test) and increased again after end of treatment ($p = 0.02$ paired t test). Treatment with the study medication (24 weeks sofosbuvir) is indicated in light grey in (A) and (B). ALT, alanine aminotransferase; ULN, upper limit of normal. (This figure appears in color on the web.)

enhance HEV replication while MMF could reduce replication *in vitro*.[14,15] In addition, the use of MMF was associated with a lower risk of developing chronic hepatitis in 1 study investigating HEV infection in heart transplant recipients.[16]

Importantly, the modest antiviral efficacy of sofosbuvir was associated with a biochemical response. The individual alanine aminotransferase (ALT) kinetics during the entire study period are displayed in Fig. 1, as values in relation to the upper limit of normal. Absolute values are shown in Fig. S2. Median ALT level decreased from baseline to week 2 from 126 U/L to 59 U/L. At week 12 and 24, ALT levels were 65 U/L and 75 U/L, respectively. ALT levels increased to baseline levels (156 U/L) 12 weeks after the end of treatment. Most patients showed a decline of ALT and aspartate aminotransferase values early during treatment. The decrease of ALT values was associated with the HEV RNA decline, but the effect on ALT remained more stable until the end of sofosbuvir treatment compared with the already increasing HEV RNA level during sofosbuvir treatment. Yet, the effect on ALT values seemed to be associated with sofosbuvir treatment because patients showed a strong rebound of ALT values to baseline values and even above in some patients after the end of therapy.

The tolerability of sofosbuvir in patients with chronic hepatitis E was generally acceptable but all patients reported at least 1 adverse event. The detailed safety analysis is shown in the supplementary information. The most frequently reported adverse events (infections) were most likely not related to sofosbuvir but rather to the immunosuppressive condition of the patients (Tables S1 and S2). However, even if sofosbuvir has been used in several thousand patients, we here emphasize a note of caution in this special setting of immunocompromised patients. The patient with common variable immune deficiency experienced an acute renal and hepatic failure and subsequently died due to sepsis. Future studies may focus first on solid organ transplant patients before advancing to rare and complex immunosuppressive conditions.

In conclusion, sofosbuvir monotherapy showed only a modest antiviral efficacy in patients with chronic hepatitis E and failed to achieve viral elimination. Sofosbuvir should not be used as monotherapy in chronic hepatitis E. Rescue treatment options for patients with chronic hepatitis E who failed ribavirin or are not eligible remain an unmet need.

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Conflicts of interest

Markus Cornberg: *Gilead Sciences (Honoraria for lectures and consulting).* Sven Pischke: *Roche Diagnostics* (*Honoraria for lectures).* Tobias Müller: *nothing to disclose.* Patrick Behrendt: *nothing to disclose.* Felix Piecha: *nothing to disclose.* Julia Benckert: *nothing to disclose.* Daniel Todt: *nothing to disclose.* Eike Steinmann *nothing to disclose.* Armin Papkalla: *nothing to disclose.* Maria von Karpowitz: *nothing to disclose.* Armin Koch: *nothing to disclose.* Ansgar Lohse: *nothing to disclose.* Svenja Hardtke: *nothing to disclose.* Michael P Manns*: nothing to disclose.* Heiner Wedemeyer*: Gilead Sciences: Honoraria for consulting and research* support*; Roche Diagnostics: Consulting* fees *on diagnostics*.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

The study was designed and protocol was written by MC, SP, PB, AP, AK, SH, MPM and HW; the study was coordinated by MC, SH, PB and HW, patients were recruited and treated by MC, SP, TM, PB, FP, JB and HW, data analysis and statistics were performed by MvK, AK, SH, MC and HW. DT and ES provided HEV sequencing data. Drafting of the manuscript were done by MC, SH, PB, SP and HW, critical revision of the manuscript was performed by all authors, administrative support: AL and MPM.

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Supplementary data

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Intrahepatocytic necroptosis is dispensable for hepatocyte death in murine immune-mediated hepatitis

Letter to the Editor:

Hundreds of millions of people globally are affected by acute or chronic hepatitis, for which there are no effective treatments. Hepatocyte death is a key element in both initiation and progression of these liver diseases. Necroptosis emerged recently as one of the possible cell death programs involved in liver damage. This pathway is a regulated modality of necrosis that occurs in the presence of caspase inhibition. It involves RIP1/3 (receptor interacting protein) kinases and a final effector, the pseudokinase MLKL (mixed lineage kinase domain-like protein).[1] Many studies have investigated the role of these necroptosis-associated proteins in different liver injury models, sometimes with conflicting results.[2] Thus, the involvement of necroptosis in different liver diseases remains controversial and a hot topic in hepatology research.

In 2016, Günther *et al.* reported that necroptosis mediates hepatocyte death in a murine model of immune-mediated hepatitis.[3] Indeed, using mice with a total ablation of *Mlkl* ($Mlkl^{-/-}$ mice) challenged by Concanavalin A (ConA), a lectin triggering severe inflammation and T-cell mediated liver injury, they found that $Mlkl^{-/-}$ mice are completely protected from ConA-induced damage compare to wild-type mice.[4] The authors concluded that the pseudokinase MLKL is an essential mediator of hepatocellular necrosis in ConA-induced hepatitis in mice.

To deepen the understanding of the role of MLKL in ConA-induced hepatitis and particularly in the hepatocyte itself, we have generated a new mouse line with conditional ablation of *Mlkl* specifically in liver parenchymal cells ($Mlkl^{LPC\text{-}KO}$ mice). As expected, these $Mlkl^{LPC\text{-}KO}$ mice have reduced levels of MLKL in the whole liver and a total absence of MLKL in isolated hepatocytes, as shown by western blotting analyses (Fig. 1A). Importantly, intravenous treatment with ConA at a dose of 12 mg/kg for 6 h, 11 h and 16 h did not reveal any differences in levels of serum transaminases and degree of necrotic damage of the liver between $Mlkl^{LPC\text{-}KO}$ mice and $Mlkl^{fl/fl}$ littermate controls (Fig. 1B and 1C). These results show that $Mlkl^{LPC\text{-}KO}$ mice are not protected from ConA-induced hepatitis demonstrating that hepatocyte-intrinsic MLKL is dispensable for ConA-induced hepatolysis in contrast to what has been advanced by Günther *et al.*[3]

The contradictory results obtained with the $Mlkl^{-/-}$ and the $Mlkl^{LPC\text{-}KO}$ mice, explained by the use of a constitute knockout model on the one hand and specific knockout model on the other hand, suggest that necroptosis occurring in other cell population(s) is involved in hepatolysis in response to ConA injection, in which immunity is essential to hepatocyte damage.

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