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immune system receptors, including RIG-I. In addition, it is also not clear whether ME3738 has anti-viral effects on genotype 1b HCV, which is the most common and most IFN-resistant genotype in Japan [14].

Recently, HCV-infected mice have been developed by inoculating HCV-infected human serum into urokinase-type plasminogen activator (uPA)-severe combined immunodeficiency (SCID) mice engrafted with human hepatocytes [15,16]. We and other groups had reported that this mouse model is useful for evaluating anti-HCV drugs such as IFN-α and anti-NS3 protease *in vivo* [17–19].

In the present study, we investigated the effects of ME3738 on HCV replication both *in vitro* and *in vivo* using the genotype 1b HCV replicon and HCV-infected human hepatocyte chimeric mice. The results demonstrate that ME3738 itself had an inhibitory effect on HCV replication, and when combined with IFN, ME3738 enhanced the anti-HCV effect of IFN by up-regulation of ISGs, such as oligoadenylate synthetase (*OAS*) 1, myxovirus resistance protein A (*MxA*), and *ISG15* in HCV replicon cells. We also showed that the combination therapy increased *OAS1*, RNA-dependent protein kinase (*PKR*) and ubiquitin specific peptidase 18 (*USP18*) expression levels, and reduced virus levels effectively without liver cell damage in human hepatocyte chimeric mice.

Material and methods

Cell culture

Cells supporting replication of the genotype 1b-derived subgenomic HCV replicon, ORN/3-5B/KE cells [20] (kindly provided by N. Kato, Okayama University, Japan) and Con-1 cells [21], were cultured in Dulbecco's modified Eagle's medium (Gibco-BRL, Invitrogen Life Technology, Carlsbad, CA) supplemented with 10% fetal bovine serum, non-essential amino acids, glutamine, penicillin, and streptomycin (complete DMEM) in the presence of G418 (300 μg/ml; Geneticin, Invitrogen, Carlsbad, CA). ORN/3-5B/KE and Con1 replicon cells (2×10^4) were seeded onto 12-well plates and incubated for 3 days with or without ME3738 (Meiji Seika Kaisha, Tokyo, Japan) [9], human IFN-α (Dainippon Sumitomo Pharma Co., Tokyo), or the combination of both drugs.

Quantitation of HCV RNA and ISG mRNAs

RNA extraction and quantitation of HCV by real-time polymerase chain reaction (PCR) were performed as described previously [19]. Briefly, RNA was extracted from mice serum, livers, or cellular lysate using SepaGene RVR (Sankojunyaku, Tokyo, Japan) and reverse transcribed with a random hexamer and a reverse transcriptase (ReverTraAce; TOYOBO, Osaka, Japan) according to the instructions provided by the manufacturer. Quantitation of HCV RNA was performed using the Real-Time PCR system (Applied Biosystems, Foster City, CA). The primers used for amplification were 5′-GAGTGTCGTGCAGCCTCCA-3′ and 5′-CACTCGCAAGCAC CCTATCA-3′. Quantitation of ISGs (*OAS1*, *MxA*, *PKR*, *USP18* and *ISG15*) was performed using real-time PCR Master Mix (TOYOBO) and TaqMan Gene Expression Assay primer and probe sets (PE Applied Biosystems, Foster City, CA). Thermal cycling conditions were as follows: a precycling period of 1 min at 95 °C followed by 40 cycles of denaturation at 95 °C for 15 s and annealing/extension at 60 °C for 1 min. Each ISG expression level was expressed relative to the endogenous RNA levels of the housekeeping reference gene glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*).

Luciferase reporter assay

After 72 h of IFN and/or ME3738 treatment, ORN/3-5B/KE cells were harvested with Renilla lysis reagent (Promega, Madison, WI) and subjected to the luciferase assay according to the manufacturer's protocol.

Western blotting

The cells were ruptured with 250 μl lysis buffer [10 mM Tris/HCl pH 7.4, 140 mM NaCl and 0.5% (v/v) NP-40] followed by centrifugation for 2 min at 15,000g. Cell lysates were subjected to Western blotting using antibodies against NS3 (Novocastra Laboratories, UK) and β-actin (Sigma, Tokyo, Japana) as described previously [22].

WST assay

Cell viability was determined by employing tetrazolium salt, WST-8, using the WST-8 Cell Proliferation Assay Kit (Dojindo Laboratories., Kumamoto, Japan), according to the instructions provided by the manufacturer.

Human serum samples

Human serum samples containing high titers of genotype 1b HCV (2.2×10^6 copies/ml) were obtained from a patient with chronic hepatitis after obtaining written informed consent. Aliquots were stored in liquid nitrogen until use.

Animal treatment

All animal protocols in this study were in accordance with the guidelines of the local committee for animal experiments and under approval of the Ethics Review Committee for Animal Experimentation of the Graduate School of Biomedical Sciences, Hiroshima University. We transplanted human hepatocytes into uPA$^{+/+}$/SCID$^{+/+}$ mice as described previously [16]. All mice used in this study were transplanted with frozen human hepatocytes obtained from the same donor. Mice were injected intravenously with 50 μl of HCV-positive human serum samples. Six weeks after HCV infection, mice were fed a normal chow containing 0.15% (w/w) ME3738 for 4 weeks, with or without IFN-α. IFN-α-treatment was provided daily by intramuscular injection of diluted IFN solution. Serum samples were collected every week, and human serum albumin (HSA) concentration and HCV RNA were measured. Mouse serum concentrations of HSA, which correlate with the repopulation rates, were measured as described previously [16]. Serum ME3738 concentrations were measured by liquid chromatography/mass spectrometry/mass spectrometry. After the fourth week of treatment, mice were sacrificed, and livers were either fixed with 4% buffered-paraformaldehyde for histological examination or frozen immediately in liquid nitrogen to measure HCV core antigen. To investigate the expression of ISGs in mouse livers, mice were kept for 1 week with or without 0.45% (w/w) ME3738 and then given a single injection of 1500 IU/g IFN-α. Four hours after injection, mice were sacrificed and liver samples were collected.

Quantitation of HCV core antigen in the mouse liver

Livers were homogenized in phosphate-buffered saline with 1% Triton X-100, 0.1% SDS, and 0.5% sodium deoxycholate. The homogenates were centrifuged at 20,000g for 30 min. HCV core antigen levels in the supernatant of liver homogenates were measured using enzyme immunoassay as described previously [23].

Statistical analysis

All data are expressed as mean ± SD. Levels of HCV RNA and ISG mRNAs were compared using the Mann–Whitney *U*-test. A *p* value less than 0.05 was considered statistically significant. All statistical analyses were performed with SPSS 14.0 software (SPSS, Tokyo, Japan).

Results

Antiviral activity of ME3738 on HCV subgenomic replicon

The effect of ME3738 on HCV replication was analyzed *in vitro* using subgenomic HCV replicon cells possessing the luciferase reporter. ORN/3-5B/KE cells were treated with either IFN-α or ME3738 for 72 h. The luciferase reporter assay demonstrated that the HCV RNA replication level decreased depending on the
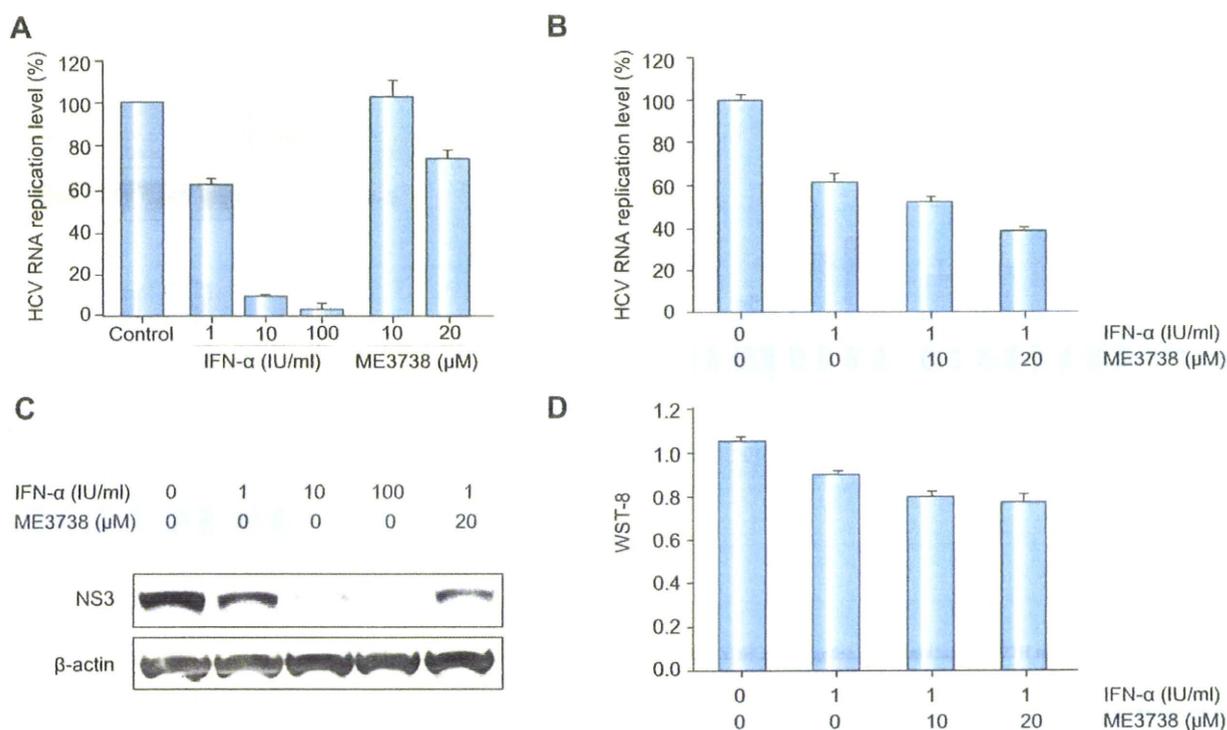
Fig. 1. Effects of ME3738 on HCV replication in the subgenomic HCV replicon, ORN/3-5B/KE cells. ORN/3-5B/KE cells were treated for 72 h with the indicated concentration of interferon (IFN)-α alone, ME3738 alone, or IFN-α plus ME3738. (A and B) Intracellular HCV RNA replication levels were determined as luciferase activity and expressed relative to cellular viability. (C) Cell lysates were analyzed by immunoblotting with antibodies to NS3 and β-actin. (D) Cellular viability was analyzed by WST assay. Data are represented as the mean ± SD of 6 experiments. Control: cells treated with neither ME3738 nor IFN-α.

IFN-treatment dose as reported previously (Fig. 1A) [20]. Treatment with 20 μM of ME3738 also reduced HCV RNA replication. Next, we investigated whether ME3738 enhances the effect of IFN-α. IFN-α (1 IU/ml) plus ME3738 inhibited the HCV RNA replication dose in a dependent manner with ME3738 (Fig. 1B). The level of cellular HCV NS3 protein was reduced depending on IFN-α-treatment and was reduced effectively by IFN-α/ME3738 combination treatment (Fig. 1C). The viability of cells treated with IFN-α/ME3738 combination treatment was lower than that of the control treatment and almost the same as with IFN-α treatment alone (Fig. 1D).

The effect of ME3738 was also tested in a different replicon system, Con-1 cells. ME3738 reduced HCV RNA replication dose dependently in Con-1 cells (Fig. 2A). Similar to ORN/3-5B/KE cells, IFN-α (1 IU/ml) plus ME3738 inhibited HCV RNA replication dose in a dependent manner with ME3738 (Fig. 2A), and the level of cellular HCV NS3 protein was reduced effectively by IFN-α/ME3738 combination treatment (Fig. 2B). The viability of cells treated with IFN-α/ME3738 combination treatment was lower but was not significant with IFN-α treatment alone (Fig. 2C). These results indicate that ME3738 itself has an inhibitory effect on HCV replication and enhances the effect of IFN-α.

Expression of ISGs in ME3738-treated replicon cells

We measured the levels of ISGs in drug-treated ORN/3-5B/KE cells and Con1 cells. IFN-α treatment significantly increased the expression levels of *OAS1*, *MxA*, *PKR*, *USP18* and *ISG15*, which

reached maximum levels at 24 h in ORN/3-5B/KE cells (Fig. 3A) and 8 h in Con1 cells (Fig. 3B). ME3738 treatment alone significantly increased the expression of *OAS1* in both cells. IFN-α treatment significantly increased the expression of ISGs; however, IFN-α/ME3738 combination treatment significantly induced the expressions of *OA1S*, *MxA* and *ISG15* to levels higher than IFN-α alone in both cells. These results indicate that ME3738 enhances the effect of IFN-α to increase ISG expression, and this effect may contribute to the inhibition of HCV replication.

Effect of ME3738 on HCV replication in vivo

To further analyze the effects of ME3738, we used genotype 1b HCV-infected human hepatocyte chimeric mice [17,19]. Six weeks after HCV infection, when the mice developed stable viremia (10^6–10^7 copies/ml, data not shown), the animals were treated with ME3738 alone, IFN-α alone, or ME3738/IFN-α for 4 weeks (Fig. 4A). Mouse serum concentrations of ME3738 increased in ME3738- and ME3738 plus IFN-α-treated mice (Table 1). ME3738 alone did not reduce the levels of HCV RNA in mice, while IFN-α-treatment reduced the HCV RNA levels, as reported previously [17]. ME3738 plus IFN-α-treatment significantly reduced HCV to levels lower than that achieved by ME3738 or IFN-α alone. We also measured the HCV core protein level in the livers of treated mice. As shown by replicon experiments, core protein levels were reduced most effectively by the ME3738/IFN-α-combination therapy (Fig. 4B). Since the level of HSA did not decrease in these treatments, it was concluded that

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A

B

IFN-α (IU/ml)	0	1	10	100	1
ME3738 (μM)	0	0	0	0	20

NS3

β-actin

C
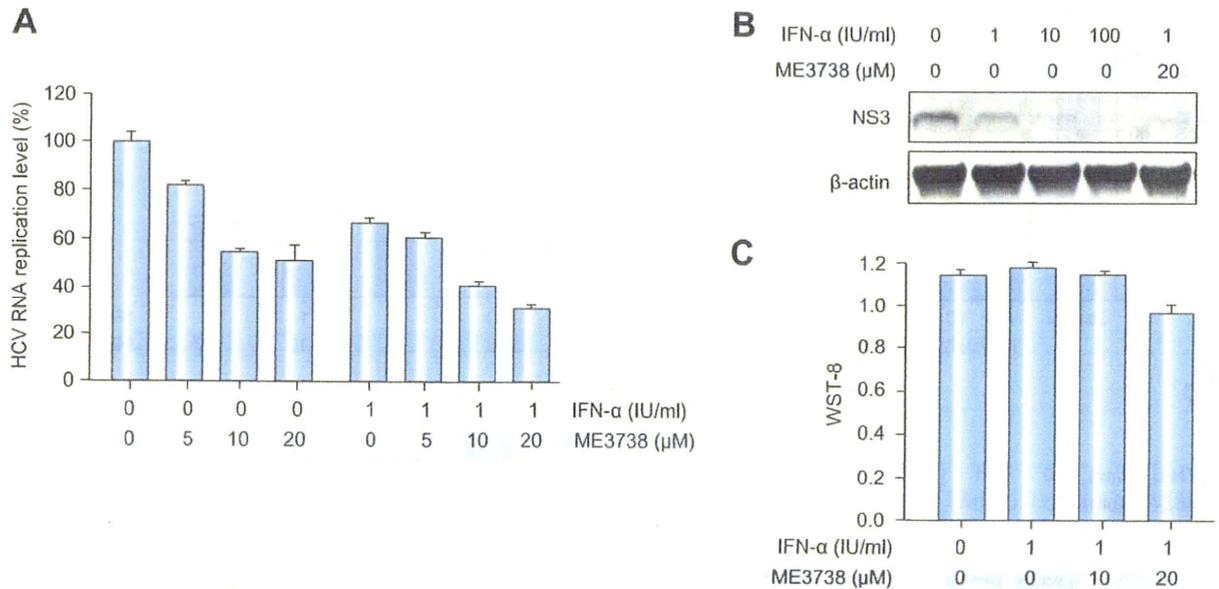
Fig. 2. **Effects of ME3738 on HCV replication in the subgenomic HCV replicon, Con1 cells.** Con1 cells were treated for 72 h with the indicated concentration of ME3738 alone or IFN-α plus ME3738. (A) Intracellular HCV RNA replication levels were determined via real-time PCR. (B) Cell lysates were analyzed by immunoblotting with antibodies to NS3 and β-actin. (C) Cellular viability was analyzed by WST assay. Data are represented as the mean ± SD of 6 experiments. Control: cells treated with neither ME3738 nor IFN-α.

the reduction of HCV in chimeric mice was not due to toxicity of the drugs (Fig. 4A). This was also supported by histopathological findings, including lack of cytotoxic changes in the livers of all four groups of mice (Fig. 4C). The effect of ME3738 to increase ISG expression was assessed in mouse liver following treatment with a high concentration of ME3738 for 1 week and a single injection of IFN-α. ME3738 alone showed no increase in the expression of ISGs in mouse livers (Fig. 5). IFN-α treatment significantly increased the expression of ISGs; however, IFN-α/ME3738 combination treatment significantly induced the expressions of *OAS1*, *PKR* and *USP18* mRNA levels in mouse livers to levels higher than IFN-α alone. These results indicate that ME3738 inhibits HCV replication, enhancing the effect of IFN-α to increase ISG expression *in vivo*.

Discussion

Although the treatment outcome of chronic HCV infection has improved with the advent of pegylated IFN-α and ribavirin, the eradication rate of HCV is only about 50%. Many patients are unable to receive this therapy because of the harmful side effects or the financial costs. Development of effective, safe and inexpensive therapies should be encouraged.

ME3738 is reported to attenuate various liver pathologies in animals [8–12]. Furthermore, Hiasa et al. reported recently that ME3738 induces IFN-β mRNA expression and inhibits the replication of HCV [13]. We thus attempted in this study to evaluate the effect of ME3738, especially in combination with IFN-α, on HCV.

The results of the present study show that ME3738 induced the gene expression of *OAS* (Fig. 2) and inhibited HCV replication (Fig. 1A). Hiasa et al. reported that ME3738 enhanced the expression of *IFN-β* mRNA and that the enhanced production of IFN-β

resulted in the increased expression of ISGs [13]. They showed also that the effect of ME3738 on HCV was abolished following the inhibition of IFN-β expression with siRNA or antibody. Our results are consistent with their findings. The extent of the increase in ISG expression was smaller in Hiasa et al. [13] than in our results. This is probably because they used the T7-genotype 1a-cDNA transient transfection-infection system to produce HCV in HepG2 or Huh7 cells [13,24,25] and assessed the effect of ME3738 by utilizing naturally produced IFN-β. The amount of IFN is likely to be very small in their system compared to that used in our study. We also tried to detect *IFN-β* mRNA in our replicon system but were unable to detect it in our replicon cells (Huh7 based ORN/3-5B/KE cells and Con1 cells). This is probably due to a defect of the innate immune system in producing IFN-β in those cells. This is consistent with their finding that ME3738 had an inferior effect in Huh7 cells than in HepG2 cells to produce ISG products [13].

As we showed in this study, ME3738 enhances the effect of IFN against HCV replication both *in vitro* (Figs. 1B and 2A) and *in vivo* (Figs. 4A and 4B). ME3738 enhanced the effect of IFN-α by increasing the expression levels of ISGs both *in vitro* (Fig. 3) and *in vivo* (Fig. 5). How ME3738 enhances the transcription of ISGs is unknown at this stage. ME3738 was reported initially to protect liver cells against injury through induction of IL-6 [8,9]. IL-6 is reported to provide protection to certain cells [26–28] by preventing apoptosis. In the present study, we tried to detect IL-6 protein in the serum and mRNA in the liver of ME3738-treated mice. However, the levels of both were too low to measure. Further studies should be conducted to elucidate the mechanism by which ME3738 enhances immunity against viral infections.

Our results showed that ME3738 did not reduce cell viability. We also showed that the drug is not hepatotoxic, as inferred by HSA level and liver histology. Since ME3738 is reported to
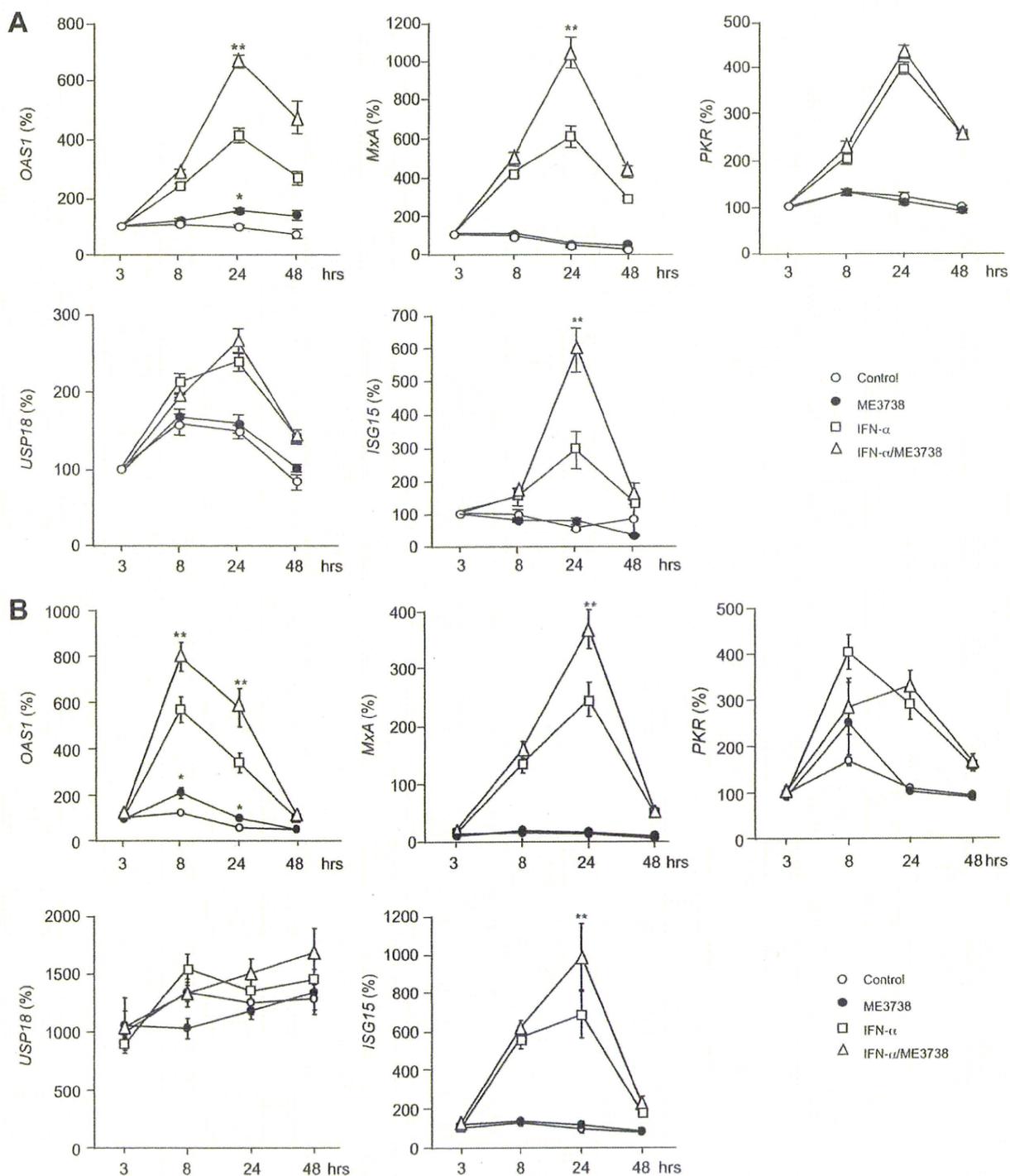
Fig. 3. **Effects of ME3738 on the expression of interferon-stimulated genes.** ORN/C-5B/KE cells (A) and Con1 cells (B) were treated with 20 μM of ME3738 and/or 1 IU/ml of interferon (IFN)-α for 48 h. Intracellular gene expression levels of oligoadenylate synthetase (*OAS*), myxovirus resistance protein A (*MxA*), double stranded RNA-activated protein kinase (*PKR*), *USP-18* and interferon-stimulated gene (*ISG*) 15 were measured at the indicated times. RNA levels were expressed relative to *GAPDH* mRNA. Data are shown as the mean ± SD of 6 experiments. Control: cells treated with neither ME3738 nor IFN-α. (*p <0.05 compared with Control, **p <0.05 compared with IFN-α treatment).

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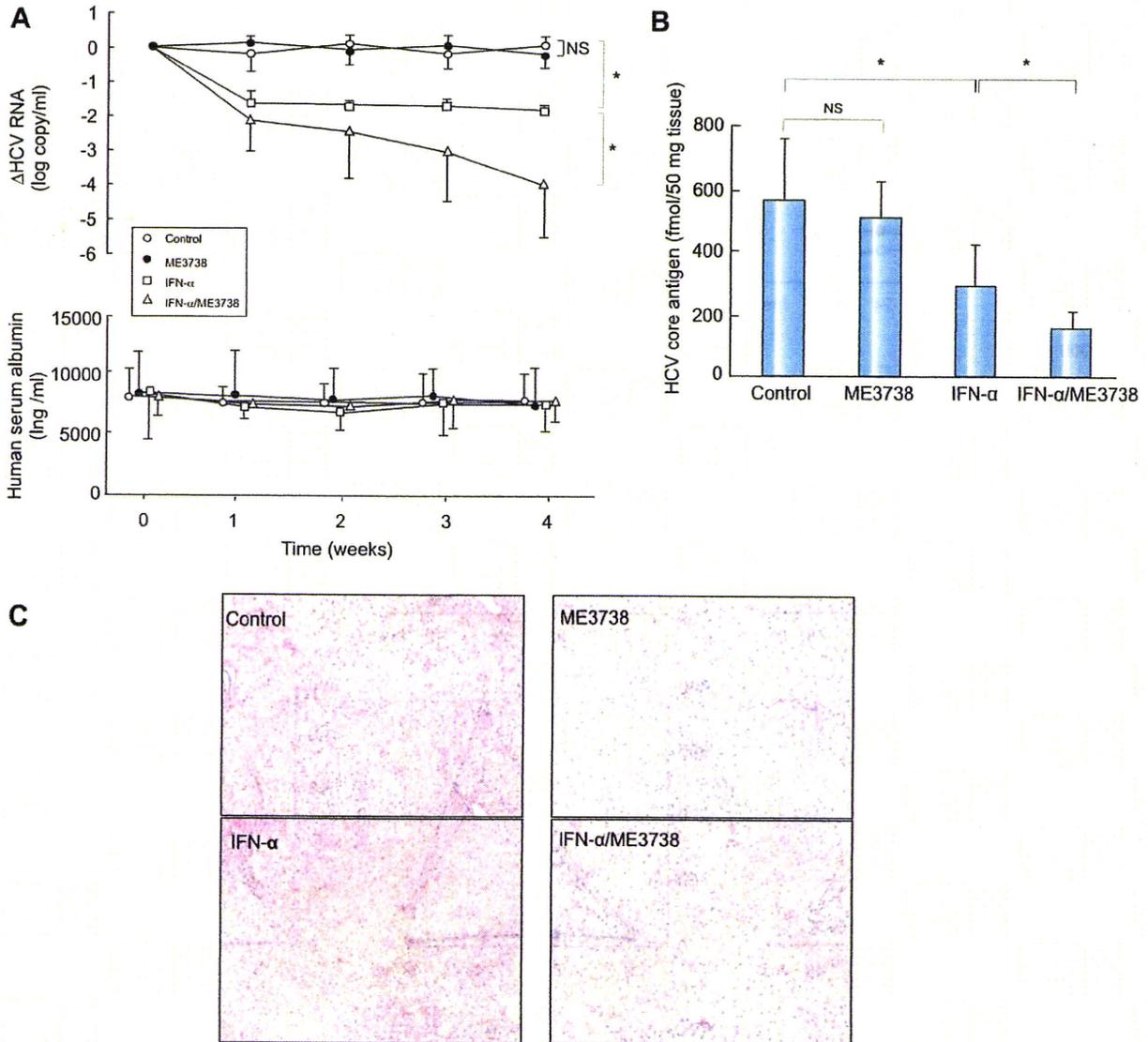
Fig. 4. ME3738 enhances the effect of IFN in mice with HCV infection. Mice were injected intravenously with 50 µl of HCV-positive human serum samples. Six weeks after HCV infection, mice were treated with ME3738 and/or interferon (IFN)-α for 4 weeks. (A) Mouse serum samples were obtained every week, and HCV RNA titer (upper panel) and human serum albumin concentration (lower panel) were analyzed. HCV core antigen was measured in the mouse livers after 4 weeks of treatment (B). Data are mean ± SD of 6 mice. (*$p < 0.05$; **$p < 0.01$; NS, not significant). (C) Liver samples obtained from mice were stained with hematoxylin-eosin (Original magnification, 100×). Note the lack of specific changes in the mice of each group. Control: HCV-infected mice treated with neither ME3738 nor IFN-α.

Table 1. Concentrations of ME3738 in mouse serum samples.

	Control	ME3738	IFN-α	ME3738/IFN-α
ME3738 (µM)	<0.01	4.02 ± 0.90	<0.01	2.44 ± 0.21

Concentrations of ME3738 in serum samples obtained from mice after 4 weeks of treatment were measured by liquid chromatography/mass spectrometry/mass spectrometry. Data are shown as mean ± SD of three mice. Control: HCV-infected mice treated with neither ME3738 nor IFN-α.

attenuate liver disease in several animal models of acute and chronic liver injury [8–12], the drug could be suitable for treatment of patients with chronic hepatitis C. In the current regimen of PEG-IFN and ribavirin combination therapy, IFN reduces the replication rate of the virus by inducing expression of ISGs in liver cells. Ribavirin enhances the effect of IFN synergistically through an unknown mechanism. ME3738 also enhances the effect of IFN similarly to ribavirin and may protect liver cells from apoptosis. Combination therapy using these three drugs might yield excellent anti-viral and anti-inflammatory effects. Alternatively, ME3738 could be used instead of ribavirin if the drug shows a superior effect in combination with IFN. Further animal and human studies should be conducted to develop an effective regimen for the treatment of patients with chronic hepatitis C.
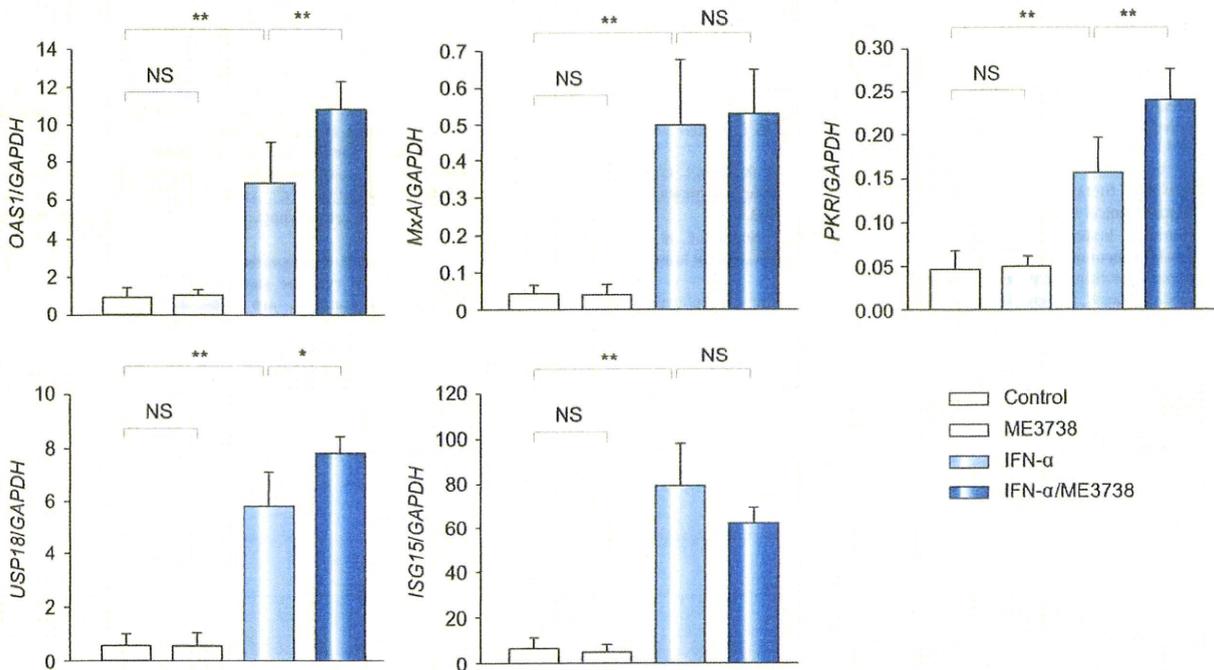
Fig. 5. Interferon-stimulated genes expression in mouse liver samples. Mice were treated with or without 0.45% (w/w) ME3738 for 1 week and then given a single injection of 1500 IU/g IFN-α. Four hours after IFN-α injection, interferon stimulated gene expression in mouse livers was measured. RNA levels are expressed relative to GAPDH mRNA. Data are presented as mean ± SD of six mice. Control: Mice treated with neither ME3738 nor IFN-α. (*p <0.05; **p <0.01; NS, not significant).

Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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Research Article

Viral Hepatitis

Elimination of hepatitis C virus by short term NS3-4A and NS5B inhibitor combination therapy in human hepatocyte chimeric mice

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Background & Aims: The current treatment regimen for chronic hepatitis C virus (HCV) infection is peg-interferon plus ribavirin combination therapy. The majority of developing therapeutic strategies also contain peg-interferon with or without ribavirin. However, interferon is expensive and sometimes intolerable for some patients because of severe side effects.

Methods: Using human hepatocyte chimeric mice, we examined whether a short term combination therapy with the HCV NS3-4A protease inhibitor telaprevir and the RNA polymerase inhibitor MK-0608 with or without interferon eradicates the HCV from infected mice. The effect of telaprevir and MK-0608 combination therapy was examined using subgenomic HCV replicon cells.

Results: Combination therapy with the two drugs enhanced inhibition of HCV replication compared with either drug alone. In *in vivo* experiments, early emergence of drug resistance was seen in mice treated with either telaprevir or MK-0608 alone. However, emergence was prevented by the combination of these drugs. Mice treated with a triple combination therapy of telaprevir, MK-0608, and interferon became negative for HCV RNA soon after commencement of the therapy, and HCV RNA was not detected in serum of these mice 12 weeks after cessation of the therapy. Furthermore, all mice treated with a high dose telaprevir

and MK-0608 combination therapy for 4 weeks became negative for HCV RNA 1 week after the beginning of the therapy and remained negative after 18 weeks.

Conclusions: Eradication of HCV from mice with only 4 weeks of therapy without interferon points the way to future combination therapies for chronic hepatitis C patients.

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Introduction

Chronic hepatitis C virus (HCV) infection is a leading cause of cirrhosis, liver failure, and hepatocellular carcinoma [1,2]. The current standard treatment for patients chronically infected with HCV is the combination of peg-interferon (PEG-IFN) and ribavirin (RBV) [3–5]. However, this treatment results in a sustained viral response (SVR), defined as negative for HCV RNA 24 weeks after cessation of the therapy, in only about 50% of patients with genotype 1 HCV infection with high viral load [3–5]. In view of the lack of effectiveness of the current therapy, many molecules have been tested for development of novel anti-HCV therapies. Recently, a number of new selective inhibitors of HCV proteins, the so-called STAT-C (specifically targeted antiviral therapy for HCV) inhibitors, have been in development. The HCV NS3-4A protease inhibitor and the NS5B polymerase inhibitor, as well as an inhibitor of NS5A function, have been demonstrated to have potent anti-HCV effects and have proceeded to clinical trials [6].

Although the anti-viral effect of these drugs is quite potent, monotherapy using these drugs results in early emergence of drug-resistant strains [7,8]. Accordingly, these drugs are used in combination with PEG-IFN and RBV. However, because IFN-treatment is expensive and is frequently associated with serious adverse events, such as cytopenias, rash/itching, alopecia, and mental disorders [3–5,9], a new treatment strategy, especially one that does not use IFN, is needed for chronic hepatitis C patients.

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The immunodeficient urokinase-type plasminogen activator (uPA) mouse permits repopulation of the liver with human hepatocytes that can be infected with HCV [10]. We and other groups reported that the human hepatocyte chimeric mouse is useful for evaluating anti-HCV drugs such as IFN-alpha and the NS3-4A protease inhibitor [11–14]. In this study, we used the NS3-4A protease inhibitor telaprevir (VX950; MP424; Mitsubishi Tanabe Pharma Co., Osaka, Japan) [15] and the NS5B RNA polymerase inhibitor MK0608 (2′-C-methyl-7-deaza-adenosine) [16] and investigated the effect of a short term combination treatment with these drugs on HCV replication both *in vitro* and in *vivo*, and showed a successful elimination of viruses in HCV-infected chimeric mice without the use of IFN. Although the dose of the drugs used in this study might be intolerable in humans, elimination of the virus without IFN by only 4 weeks of therapy sheds light on approaches to developing combination therapies using multiple STAT-C agents without IFN.

Materials and methods

Cell culture

An HCV subgenomic replicon plasmid, pRep-Feo, was derived from pRep-Neo (originally, pHC-VIbneo-delS [17]). The pRep-Feo carries a fusion gene comprising firefly luciferase (*Fluc*) and neomycine phosphotransferase, as described elsewhere [18,19]. Replicon RNA was synthesized *in vitro* by T7-RNA polymerase (Promega, Madison, WI) and transfected into Huh7 cells by electroporation. Huh7 cells were maintained in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum at 37 °C under 5% CO_2. After culturing in the presence of G418 (Wako, Osaka, Japan), cell lines stably expressing the replicons were established (Huh7/Rep-Neo).

Luciferase assay

Replicon cell lines were treated with various concentrations of either telaprevir or MK-0608 for 72 hrs, and HCV RNA replication level was quantified by internal luciferase assay. Luciferase activities were quantified using a luminometer (Lumat LB9501; Promega) and the Bright-Glo Luciferase Assay System (Promega). The 50% inhibitory concentrations (IC_{50}) were defined as the drug concentrations producing a 50% reduction in the levels of luciferase activities relative to average levels in untreated cultures.

MTT assays

Cell viability was measured under the same experimental settings using a tetrazolium (MTT)-based viability assay (BioAssay, California, USA) according to the manufacturer's directions. The 50% cytotoxic concentrations (CC_{50}) were defined as the drug concentrations producing a 50% reduction in absorbance relative to the average level in untreated cultures.

Animal treatment

Generation of the uPA$^{+/+}$/SCID$^{+/+}$ mice and transplantation of human hepatocytes were performed as described recently by our group [20]. All mice were transplanted with frozen human hepatocytes obtained from the same donor. All animal protocols described in this study were performed in accordance with the guidelines of the local committee for animal experiments, and all animals received humane care. Infection, extraction of serum samples, and sacrifice were performed under ether anesthesia. Mouse serum concentrations of human serum albumin (HSA), correlated with the repopulation index [20], were measured as previously described [21]. Eight weeks after hepatocyte transplantation, mice were intravenously injected with 100 μl of HCV-positive human serum samples. Mice serum samples were obtained every one or 2 weeks after HCV infection, and HSA and HCV RNA levels were measured.

Treatment with anti-HCV drugs in HCV-infected mice

Telaprevir and MK-0608 were dissolved with a specific solvent. Eight weeks after HCV infection when the mice developed stable viremia (10^6 to 10^9 copies/ml), mice were administered either 200 mg/kg of telaprevir or 3–50 mg/kg of MK-0608 orally twice a day for 4 weeks. The specific solvent had no anti-HCV effect in this mouse model (data not shown). To analyze the effect of the combination treatment with telaprevir and MK-0608, these drugs were mixed and given together as a cocktail. Human IFN-alpha-treatment was provided daily by intramuscular injection of diluted IFN solution (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) for 4 weeks.

Human serum sample

Human serum containing a high titer of genotype 1b HCV (2.2×10^6 copies/ml) was obtained from a patient with chronic hepatitis who had provided written informed consent to participate in the study. Serum samples were divided into small aliquots and stored in liquid nitrogen until use. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the institutional review committee.

RNA extraction and amplification

RNA extraction, nested PCR and quantitation of HCV by real-time polymerase chain reaction (PCR) were performed as described previously [12,13]. Briefly, RNA was extracted from serum samples and extracted livers using SepaGene RVR (Sankojunyaku, Tokyo, Japan) and reverse transcribed with a random hexamer and a reverse transcriptase (ReverTraAce; TOYOBO, Osaka, Japan) according to the instructions provided by the manufacturer. Quantitation of HCV cDNA was performed using Light Cycler (Roche Diagnostic, Japan, Tokyo). The lower detection limit of real-time PCR is 10^3 copies/ml.

Sequence analysis

The nucleotide and amino acid sequences of the NS3 and NS5B region of HCV were determined by direct sequencing following PCR amplification of cDNA after reverse transcription of HCV RNA. The primers used to amplify the NS3 region were 5′-GTGCTCCAAGCTGGCATAAC-3′ and 5′-AGGACCGAGGAATCGAACAT-3′ as the first (outer) primer pair and 5′-CTAGAGTGCCGTACTTCGTG-3′ and 5′-ACTGATCCTGGAGGCGTAGC-3′ as the second (inner) primer pair. The primers used to amplify the NS5B region were 5′-TAAGCGAGGAGGCTGGTGAG-3′ and 5′-CCTATTGGCCTGGAGTGTTT-3′ as the first (outer) primer pair and 5′-GACTCAACGGTCACTGAGAG-3′ and 5′-CCTATTGGCCTGGAGTGTTT-3′ as the second (inner) primer pair. PCR was performed in a 25 μl solution, consisting of a reaction buffer (12.5 μl, 2× PCR buffer for FOD FX), 5 μl 2 mM dNTPs, 0.75 μl F primer (10 μM), 0.75 μl R primer (10 μM), 1 μl Temp DNA (10 pg–200 ng), 0.5 μl KOD FX, 4.5 μl D.W. RT-PCR reactions were carried out following the manufacturer's instructions (Biometra T-Personal; Montreal Biotech Inc., Kirkland, QC, Canada). Amplification conditions included an initial denaturation at 94 °C for 2 min, 35 cycles of amplification (denaturation at 94 °C for 2 min, annealing of primer at 56 °C (1st PCR) or 59 °C (2nd PCR) for 30 s; extension at 68 °C for 2 min 30 s (NS3, 1st PCR), 1 min 30 s (NS3, 2nd PCR), 2 min (NS5B, 1st PCR), or 1 min 10 s (NS5B, 2nd PCR)); and final extension at 68 °C for 5 min.

Results

Anti-viral activity of telaprevir and MK-0608 on HCV subgenomic replicon cells

The effect of telaprevir and MK-0608 on HCV replication was analyzed *in vitro* using HCV replicon cells. Huh7/Rep-Feo cells were treated with various concentrations of either telaprevir or MK-0608. Measured luciferase activity demonstrated that both drugs inhibited HCV replication in a dose-dependent manner (Fig. 1). The IC_{50} of telaprevir and MK-0608 was 0.53 and 0.51 μM, respectively, consistent with previous reports [7,16]. When

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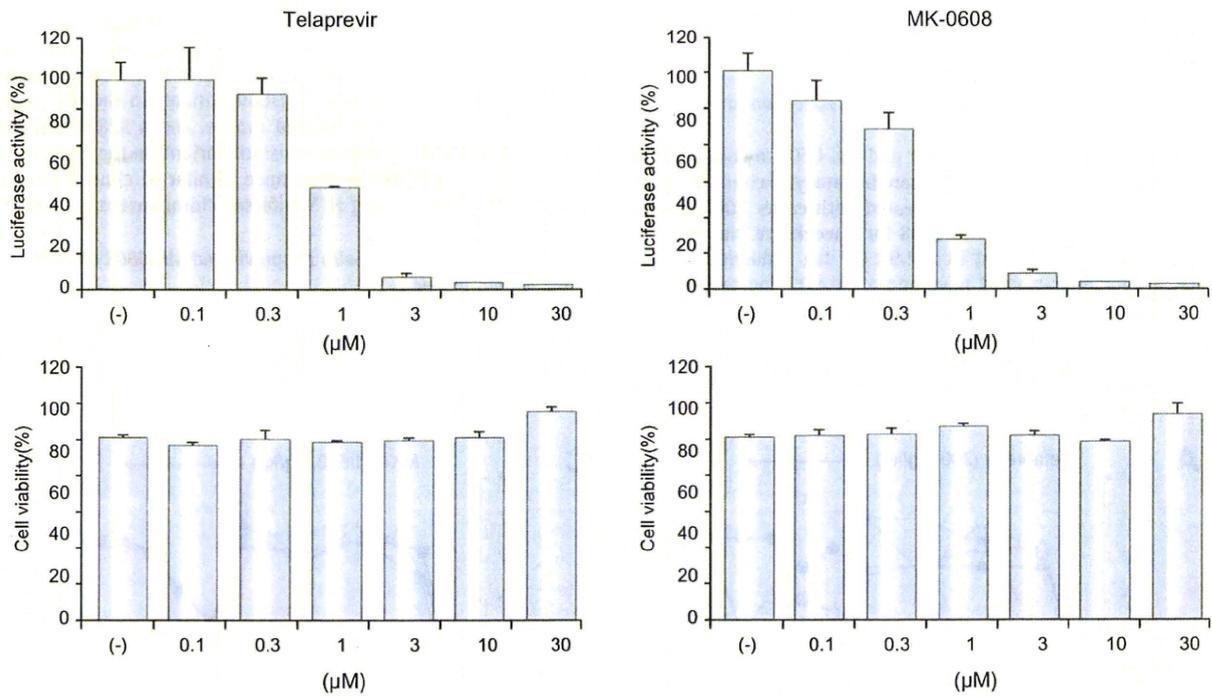
Fig. 1. *In vitro* analysis of susceptibility of HCV replicon cells to anti-HCV drugs. Huh7/Rep-Neo cells were treated for 72 h with the indicated concentrations of either telaprevir or MK-0608. Intracellular HCV RNA replication levels were determined as luciferase activities (upper panel), and expressed relative to cellular viabilities (lower panel). Bars represent means ± SD of three experiments.
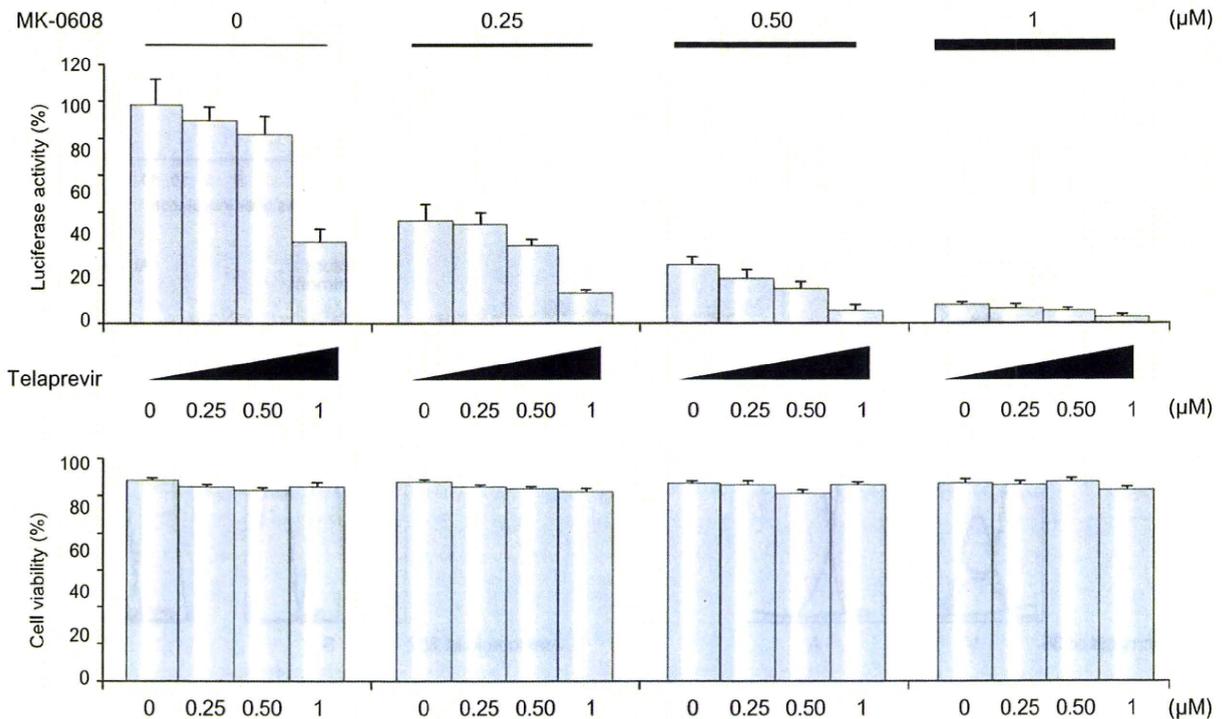
Fig. 2. *In vitro* analysis of susceptibility of HCV replicon cells to combination treatment with anti-HCV drugs. Huh7/Rep-Neo cells were treated for 72 h with the indicated concentration of MK-0608 plus telaprevir. Intracellular HCV RNA replication levels were determined as luciferase activities (upper panel), and expressed relative to cellular viabilities (lower panel). Bars are means ± SD of 3 experiments.

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telaprevir and MK-0608 were combined, the anti-HCV effect was increased without cellular damage (Fig. 2).

Effects of telaprevir and MK-0608 on HCV replication in vivo

To analyze the effect of telaprevir and MK-0608 *in vivo*, we used genotype 1b HCV-infected human hepatocyte chimeric mice. Eight HCV-infected mice were treated with either 200 mg/kg of telaprevir or 3 mg/kg of MK-0608 for 4 weeks. At the end of 1 week, treatment resulted in a 1.9 ± 0.7 log reduction of HCV RNA in telaprevir-treated mice and a 2.6 ± 0.2 log reduction in MK-0608-treated mice (Fig. 3A and C). During the treatment, the level of HSA did not decrease. Serum HCV RNA level rebounded in one of the four telaprevir-treated mice and in two

of the three MK-0608-treated mice (a MK-0608-treated mouse died after 1 week of treatment). Nucleotide and amino acid sequence analysis showed the emergence of a V36A mutation (NS3-4A protease inhibitor-resistant variant) in the NS3 region (Fig. 3B) in a telaprevir-treated mouse, and a S282T mutation (NS5B polymerase inhibitor-resistant variant) in the NS5B region (Fig. 3D) in MK-0608-treated mice, similar to clinical observations and analysis using HCV-infected chimpanzees [22,23].

Combination treatment with telaprevir and MK-0608 on HCV replication in vivo

Because mono-therapy with either telaprevir or MK0608 resulted in emergence of drug-resistant variants, we analyzed the effect of
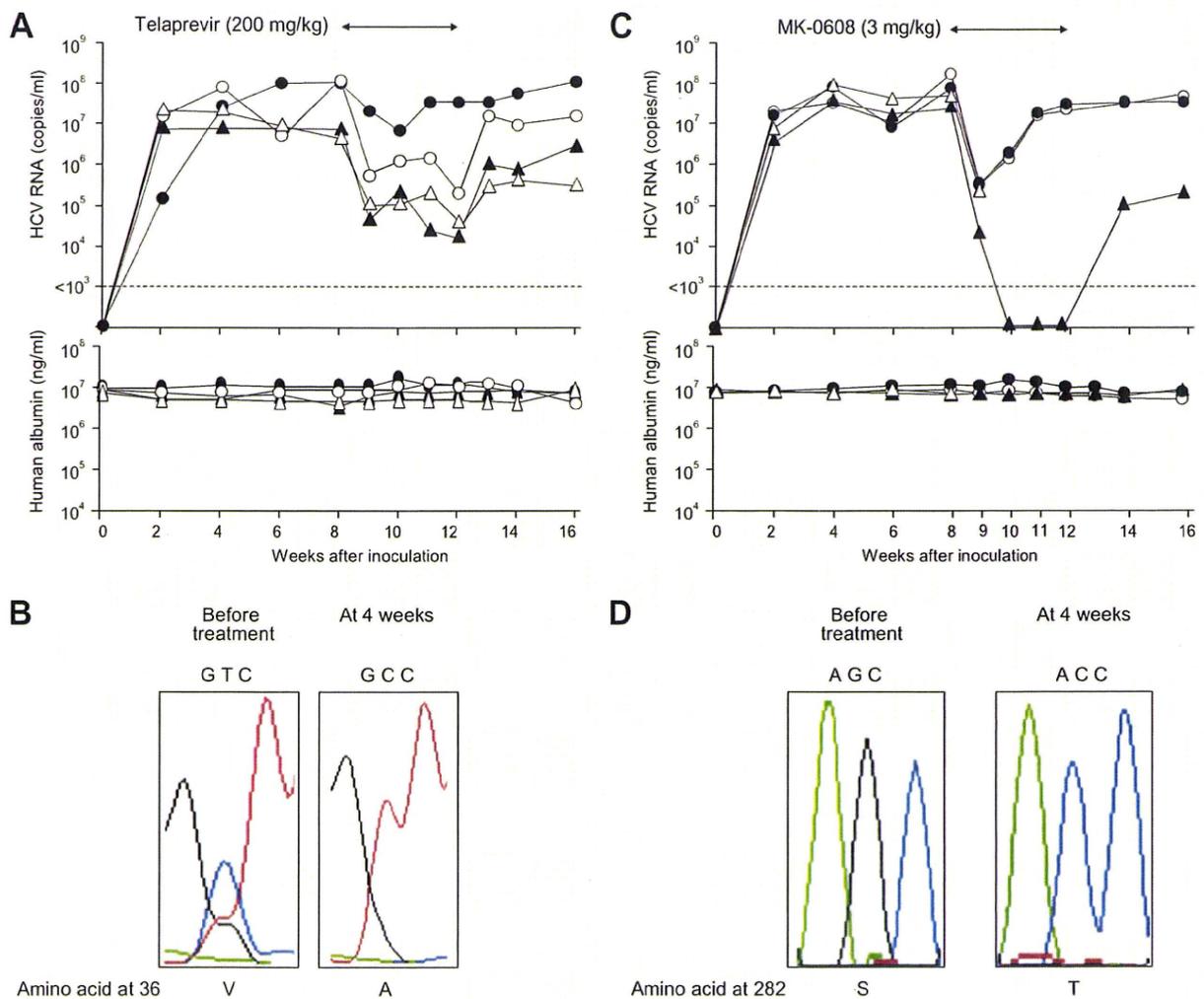
Fig. 3. Antiviral effects of either telaprevir or MK0608 monotherapy on HCV-infected mice. Mice were injected intravenously with 100 μl of HCV-positive human serum samples. Eight weeks after HCV infection, mice were treated with either 200 mg/kg of telaprevir (A) or 3 mg/kg of MK-0608 (C) for 4 weeks. Mice serum samples were obtained at the indicated times, and HCV RNA titer (upper panel) and human serum albumin concentration (lower panel) were analyzed. The horizontal dashed line represents the detection limit (10^3 copies/ml). Note that one telaprevir-treated mouse (A, closed circle) and two MK-0608-treated mice (B, closed circle and open circle) showed a viral breakthrough during the dosing period. Nucleotide and amino acid (aa) sequence analysis of aa 36 in the HCV NS3 (B) or at aa 282 in the NS5B region (D) by direct sequencing in mice serum samples obtained before treatment and at 4 weeks.
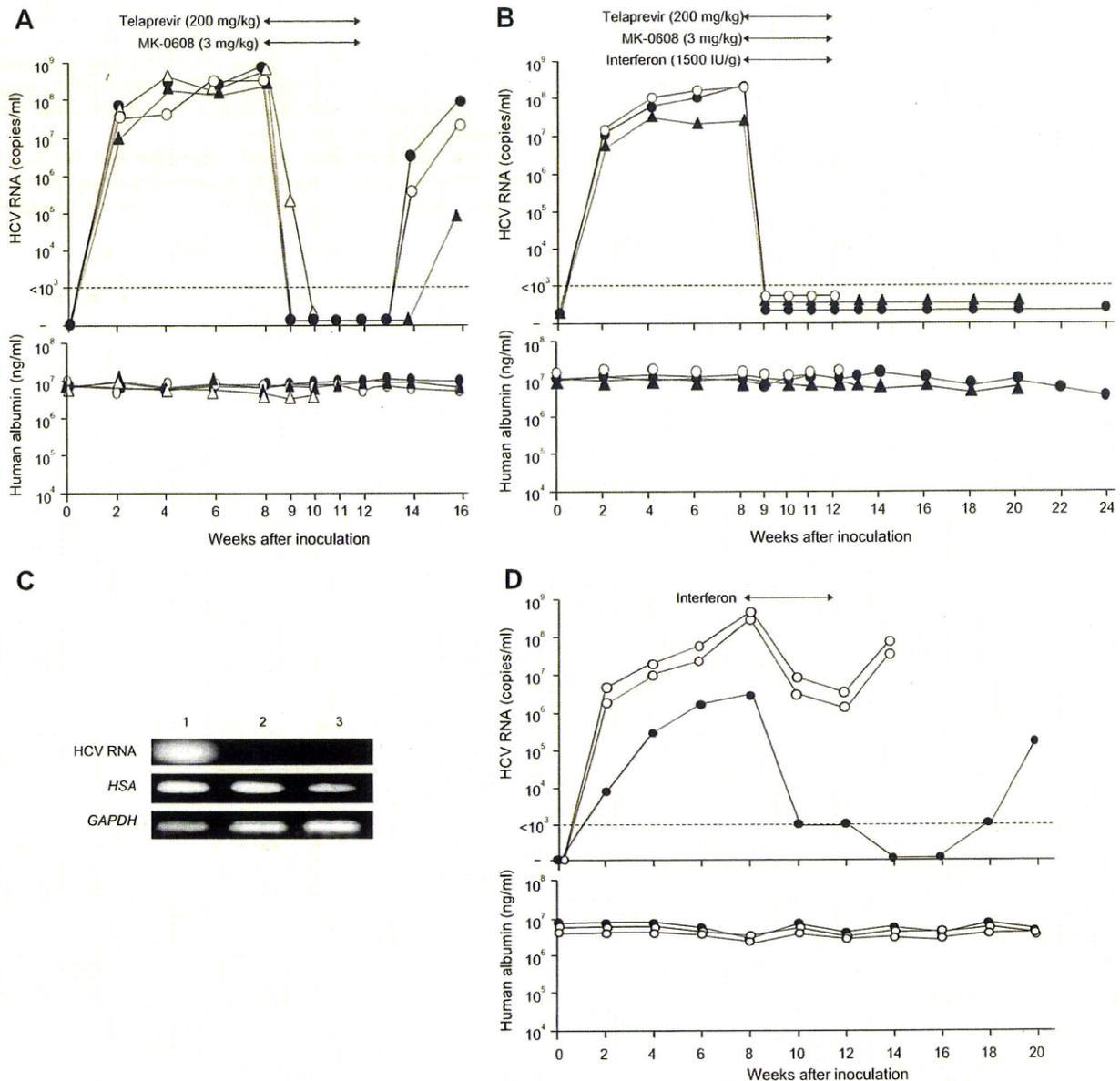
Fig. 4. Antiviral effect of combination treatment on HCV-infected mice. HCV-infected mice were treated with 200 mg/kg of telaprevir plus 3 mg/kg of MK-0608 without (A) or with (B) 1500 IU/g of human interferon-alpha for 4 weeks. Mice serum samples were obtained at the indicated times, and HCV RNA titer (upper panel) and human serum albumin concentration (lower panel) were analyzed. (C) Nested PCR of HCV RNA, human serum albumin (*HSA*) and *GAPDH* in a telaprevir, MK-0608 and interferon-alpha-treated mouse liver at 24 weeks (lane 2). Mice livers with (lane 1) or without (lane 3) HCV-infection were also analyzed. (D) HCV-infected mice were treated with either 1500 (open circles) or 7000 IU/g (closed circles) of interferon-alpha for 4 weeks.

combination treatment of these drugs with or without IFN on HCV replication *in vivo*. Four HCV-infected mice were treated with telaprevir plus MK-0608 for 4 weeks (Fig. 4A). Serum HCV RNA became negative by nested PCR with this combination treatment in all mice. One mouse died after 2 weeks of treatment. During the treatment, no emergence of resistant strains was observed in each of the remaining three mice; however, all mice became positive for HCV RNA again after cessation of the therapy. Another three mice were treated with telaprevir, MK-0608 and IFN-alpha for 4 weeks (Fig. 4B). HCV RNA became undetectable

in all three mice 1 week after the beginning of the therapy. After 4 weeks of treatment, one mouse died. In the remaining two mice, HCV RNA did not become positive after cessation of the therapy. One of the remaining two mice died at 20 weeks, and the remaining mouse was sacrificed at 24 weeks (12 weeks after the cessation of therapy). HCV was probably eliminated because no HCV RNA was detected by nested PCR in this mouse liver (Fig. 4C). As a control, HCV-infected mice were treated with 1500 IU/g/day of IFN-alpha alone for 4 weeks, resulting in a two log reduction (Fig. 4D). HCV RNA became undetectable with

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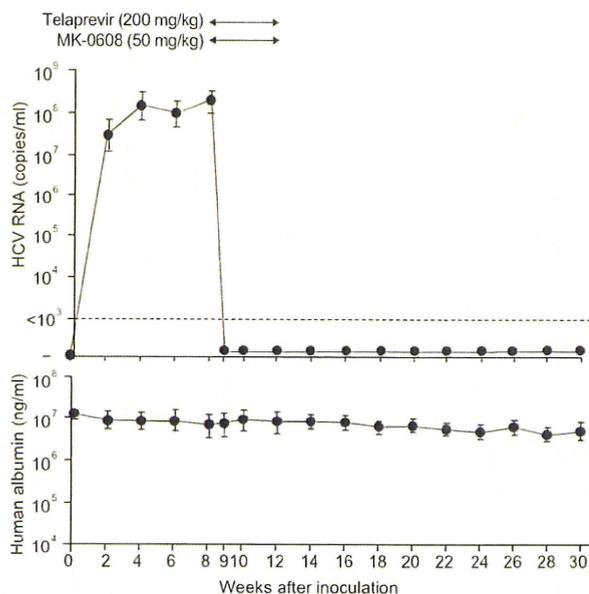
Fig. 5. High doses of MK-0608 and telaprevir combination treatment eliminates virus in HCV-infected mice. HCV-infected mice were treated with 50 mg/kg of MK-0608 and 200 mg/kg of telaprevir for 4 weeks. Mice serum samples were obtained at the indicated times, and HCV RNA titer (upper panel) and human serum albumin concentration (lower panel) were analyzed. Points represent the means ± SD of five mice.

administration of 7000 IU/g/day of IFN-alpha treatment. However, the virus rebounded after cessation of the therapy.

Four-week high dose combination therapy of MK-0608 and telaprevir eliminated HCV from mice

We investigated whether combination treatment with high doses of MK-0608 and telaprevir without IFN eliminates viruses from HCV-infected mice. Five HCV-infected mice were treated with high doses of MK-0608 (50 mg/kg) and telaprevir (200 mg/kg) for 4 weeks. Serum HCV RNA titer became undetectable 1 week after commencement of the therapy and remained undetectable in all mice at 30 weeks (18 weeks after cessation of the therapy) (Fig. 5). No apparent toxicity of the drugs was observed as none of the mice showed a decrease in the level of serum HSA.

Discussion

Since we began performing treatment experiments using human hepatocyte chimeric mice with HCV, we have administered many different drugs to analyze the effects on suppression or eradication of the virus. However, until we performed the experiments described in this study, we have never observed long term absence of the virus following cessation of the therapy [12,24]. Strikingly, after only 4 weeks of triple therapy with IFN, telaprevir and MK0608, was long term absence of the virus in mouse serum after cessation of the therapy visible (Fig. 4B). Furthermore, high dose telaprevir and MK-0608 combination therapy resulted in a similar absence of the virus for 16 weeks after cessation of therapy (Fig. 5). In this study, mice were treated with 200 mg/kg of

telaprevir twice a day, and 1 week of the treatment resulted in an approximately 2 log reduction of HCV RNA (Fig. 3A), as has been observed previously in chronic hepatitis C patients treated with 450 mg of telaprevir every 8 hrs [25]. This result suggests that approximately 1/15th of a dose in this mouse model may be equivalent to a dose in humans.

During the observation period, some mice died. We do not think that this is due to the drug regimes because the chimeric mouse is weak, and approximately 50% of mice die spontaneously at week 6 after transplantation [26].

Sustained virological response, the complete elimination of the virus from the human body, is defined as testing negative for HCV RNA in serum for more than 24 weeks after cessation of the therapy. As the chimeric mouse used in this study is a weak animal, we were unable to monitor for absence of the virus beyond 24 weeks following cessation of therapy. However, negative testing for HCV RNA in mouse liver by nested PCR (Fig. 4C) 12 weeks after cessation of the therapy strongly suggests that HCV was completely eliminated from the mouse. Of course the mouse model differs from infection in humans where the virus replicates for years in the livers of infected patients. However, results of this study suggest that we will be able to eliminate the virus in humans by treating patients with regimens similar to those used in this study.

Until recently eradication of the virus with biochemical and histological improvement in chronically infected patients has long been reported only with the use of IFN or PEG-IFN [27,28]. Recently, Suzuki et al. reported for the first time eradication of the virus from chronically infected patients without IFN [29].

Elimination of the virus without IFN is desirable due to the many serious side effects of this drug [3,5–9]. However, emergence of drug resistance is a problem, as demonstrated in this study (Fig. 3) as well as in previous studies using replicon systems and HCV-infected chimpanzees [22,23]. A recent clinical study of NS3-4A and NS5B inhibitor combination therapy has reported that 13 days of this combination treatment achieved robust antiviral suppression in chronic hepatitis C patients [30]. As no study has tested the possibility of development of double drug resistant mutants, we will have to test if long term low dose treatment with any combination of STAT-C compounds might induce emergence of multi-drug resistant strains. Furthermore, as there is no report for emergence of IFN resistant strains, regimens such as combination therapy with multiple STAT-C drugs with a small or standard amount of IFN should be tested to develop the best therapy to eradicate the virus with a minimum of side effects and costs. Our further attempts to test possible combinations in mice to determine the best combination of STAT-C drugs will give us an insight into how to develop more effective therapeutic regimens in humans.

Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

Acknowledgments

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MECHANISMS OF GASTROINTESTINAL, PANCREATIC AND LIVER DISEASES

Animal model for study of human hepatitis viruses

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Abstract

Human hepatitis B virus (HBV) and hepatitis C virus (HCV) infect only chimpanzees and
humans. Analysis of both viruses has long been hampered by the absence of a small animal
model. The recent development of human hepatocyte chimeric mice has enabled us to carry
out studies on viral replication and cellular changes induced by replication of human
hepatitis viruses. Various therapeutic agents have also been tested using this model. In the
present review, we summarize published studies using chimeric mice and discuss the merits
and shortcomings of this model.

Introduction

Hepatitis B virus (HBV) and hepatitis C virus (HCV) are patho-
gens that cause chronic infection in humans. There are 360 million
and 170 million people infected worldwide with HBV or HCV,
respectively.[1,2] Infected individuals develop acute hepatitis,
chronic hepatitis and liver cirrhosis. The viruses are also important
causative agents of hepatocellular carcinoma, especially in the
Asia–Pacific region.[3] Study of the biology and development of
therapies for each virus has long been hampered by the lack of a
small animal model that supports hepatitis virus infection. This is
probably as a result of the lack of receptor molecules necessary for
viral infection in animal liver cells.

Transgenic mice that express over-length HBV-DNA export
viral particles into the serum,[4] and such animals can be used to
evaluate antiviral agents,[5–7] as well as HBV-targeted siRNA[8].
However, the virus life cycle is not established in this model, and
it is inappropriate for studying drug-resistant HBV strains.
Accordingly, researchers attempted to transplant human hepato-
cytes into mice. The development of the trimera mouse was one
such attempt, in which human hepatocytes were transplanted
under the kidney capsule of immune-deficient mice after lethal
irradiation.[9,10] However, the number of hepatocytes that could
survive on the kidney capsule was small, and normal liver archi-
tecture was not present. Although 85% of HBV-inoculated animals
developed HBV viremia, the titer was less than 10^5 virus particles
or IU/mL.[9] Similarly, 85% of HCV-inoculated animals also devel-
oped viremia,[10] but the level of the viremia only reached 10^5/mL.

Thus, the advent of human hepatocyte transplanted uPA/scid mice
has provided the first really useful model for acute and chronic
infections of human hepatitis virus.

Human liver cell transplanted uPA/scid mice

Transgenic mice in which the urokinase gene is driven by the
human albumin promoter/enhancer were developed and shown to
have accelerated hepatocyte death and consequent chronic stimu-
lation of hepatocyte growth.[11] Transplanted rat hepatocytes
proliferated and repopulated injured livers in immunodeficient
uPA mice, which were produced by mating uPA transgenic mice
with scid mice.[12] Human hepatocytes were then transplanted into
uPA/scid mice; these cells proliferated and replaced the apoptotic
mice liver cells (Fig. 1).

Such human hepatocyte chimeric mice have been shown to be
susceptible to both HBV[16] and HCV[17] infections. Repopulation
levels by human hepatocytes have been estimated by measuring
human albumin levels in mouse serum. Replication levels of both
HBV[13] and HCV[17] were higher in mice in which the repopulation
index was higher. A unique attempt to remove mouse residual liver
cells with the herpes simplex virus type-1 thymidine kinase
(HSVtk)/ganciclovir (GCV) system failed to result in a higher
repopulation rate as a result of damage to the transplanted human
hepatocyte caused by bystander effects.[18] Despite this, mice with
livers that have been highly repopulated with human hepatocytes
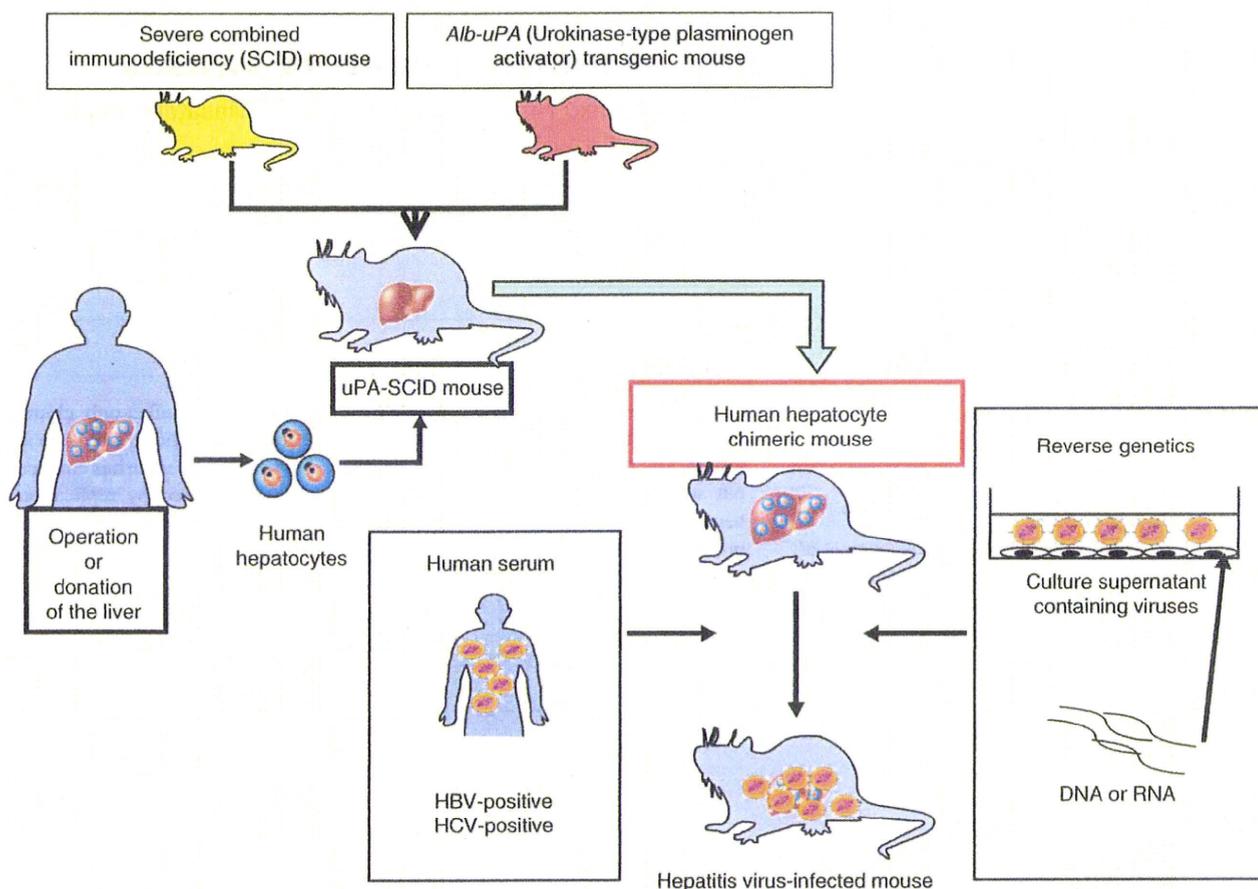
Figure 1 Generation of human hepatocyte chimeric mice and hepatitis virus infection model. A uPA/scid mouse was created by mating uPA transgenic mouse and scid mouse. Human hepatocytes obtained by surgical resection or donation were transplanted to newborn mice. The chimeric mice can be infected with hepatitis B virus (HBV) or hepatitis C (HBC) virus by injecting human serum containing these viruses. Alternatively, the mice can be infected by HBV[13] or HCV[14] created in cell culture or by injecting HCV RNA into the mouse liver.[15]

are susceptible to infection with both HBV and HCV, and as such comprised the most effective small animal model for chronic hepatitis so far developed.[19,20] An example of a highly repopulated mouse liver that we are using in experiments is shown in Figure 2.

Highly repopulated mice have been shown to be a valuable model for the study of drug metabolism.[21–29] Advances in technology for human hepatocyte transplantation have enabled serial passage of human hepatocytes in uPA/scid mice and have been shown to retain infectivity for HBV.[30]

This mouse model and other animal models for the study of hepatitis viruses have been summarized in reviews by Meuleman and Leroux-Roels,[31] Dandri *et al.*,[32,33] Barth *et al.*,[34] and Kneteman and Toso.[35] The present review will focus on key issues and updated information.

Study of hepatitis B virus infection using human hepatocyte chimeric mice

Since the initial reports of successful transmission of HBV to human hepatocyte chimeric mice in 2001 and 2004,[16,27] several researchers have reported transmission of HBV into similar

mice.[13,36,37] In these studies, passage experiments studies show that HBV replicating in mice retain infectivity.[13,36] Further, the presence of viral proteins has been shown immunohistochemically in human hepatocytes transplanted into mouse livers, but these are not present in mouse hepatocytes.[13,36,37] Formation of viral particles in infected mouse livers can be shown by electron microscopy.[36,37] Genetically engineered viruses lacking HBe-antigen have also been shown to infect chimeric mice, proving that e antigen is dispensable for viral infection and replication.[13] In contrast, HBx protein has been shown to be indispensable for viral replication.[38] Transcomplementation of HBx protein with hydrodynamic injection restored HBV infectivity in mice. Interestingly, all revertant viruses show a restored ability to express HBx.[38]

By infecting chimeric mice with genotype A, B and C, differing proliferative capacity has been shown between HBV genotypes.[37] In mice infected for a relatively short time, there are no morphological changes in HBV infected mice livers in studies.[13,36] In contrast, the occurrence of liver cell damage has been reported after long-term infection of chimeric mice with HBV[39] or with specific strains of HBV;[40] these findings are consistent with direct cytopathic effects of HBV under certain conditions.
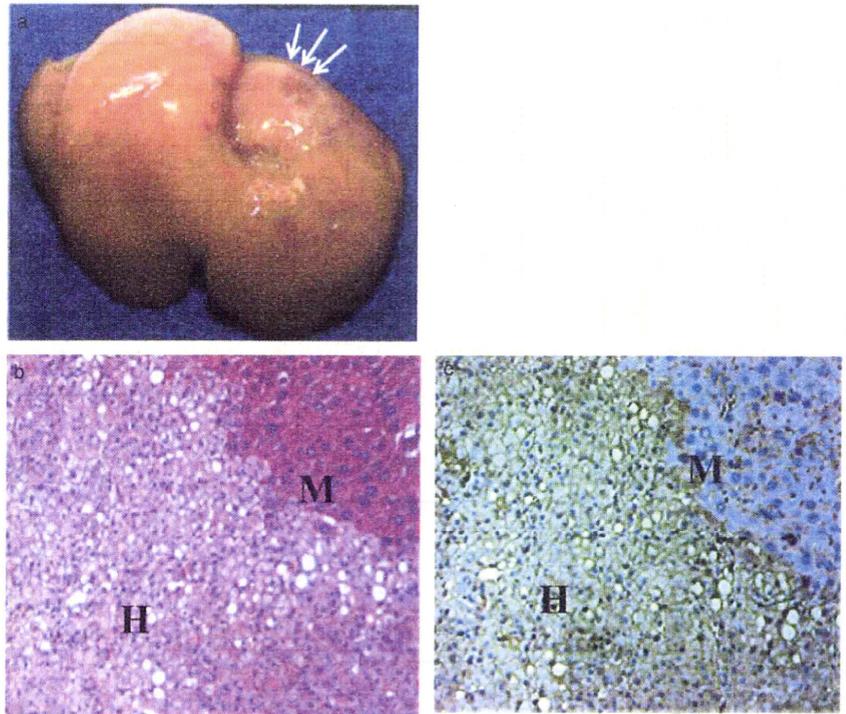
Figure 2 Representative uPA/scid mouse livers repopulated by human hepatocytes. (a) Mouse liver almost completely repopulated by human hepatocytes. Only a small portion of mouse hepatocytes are shown by arrows. (b) Microscopic figure of the mouse liver. M and H indicate regions consisting of mouse and human hepatocytes, respectively (Hematoxylin–eosin staining, magnification: ×100). (c) Microscopic figure of the mouse liver stained with antibody directed against human serum albumin.

The biological properties of a newly identified unique strain of HBV, genotype G, which replicates only in the presence of another genotype, were confirmed using the chimeric mouse.[41] Infectivity of another novel HBV strain, identified from a Japanese patient, that is divergent from known human and ape HBV has also been confirmed.[42] Titration of HBV infectivity, which previously could only be carried out using chimpanzees, can be carried out effectively using chimeric mice.[43]

Taking advantage of the absence of human immune cells in the chimeric mice, Noguchi *et al.*[44] showed that hypermutation of HBV increases in human hepatocytes under interferon treatment. Dandri *et al.* measured viral half-life in human and chimeric mice repopulated with wooly monkey hepatocytes.[45] The results clearly showed that viral half-life is shortened by immunological mechanisms in humans with low viral levels, but not in chimeric mice where functional immunity is absent. Hiraga *et al.*[46] showed an absence of interference between HBV and HCV.

Evaluation of therapeutic agents is the most important role for this mouse model. Tsuge *et al.*[13] assessed the effect of interferon and lamivudine using chimeric mice. Similarly, Dandri *et al.*[47] showed the effects of adefovir using uPA/scid mice repopulated with tupaia hepatocytes, which also support replication of human HBV. Oga *et al.*[48] identified a novel lamivudine-resistant variant that has an amino acid substitution outside of the YMDD motif. They showed that lamivudine was ineffective against the novel mutant strain. It is thus apparent that this mouse/human liver chimeric model is ideal to study the susceptibility of mutant strains to various drugs, because mutant viruses can easily be made and infected into chimeric mice.[13] The model has also been utilized to evaluate viral entry inhibitors derived from the large envelope protein.[49]

Study of hepatitis C virus using human hepatocyte chimeric mice

As observed in studies on HBV, HCV infection efficiency was poor and levels of viremia were low in mice where the repopulation rate of the mouse liver with human hepatocyte was low.[17,50] As shown in Figure 3, human albumin levels in mouse serum were significantly higher in mice in which measurable viremia developed (Hiraga *et al.* unpublished data). Recent studies have therefore been carried out using highly repopulated mice. The usefulness of a newly developed HCV assay,[51] and infectivity of a newly identified intergenotypic recombinant strain,[52] have been reported using the chimeric mice.

Using the remarkable replication ability of the JFH1 genotype 2a strain,[53] infectivity of JFH1 or intergenotypic chimeric viral particles, previously shown in cell culture, has now been shown to be infectious in chimeric mice.[54–56] Infectivity of viruses that were replicated in chimeric mice in cell culture has also been shown, and virus fitness has been studied.[55,56] The role of the HCV core+1 open reading frame and core *cis*-acting RNA elements has also been examined using the chimeric virus.[57] These elegant studies have the limitation that the non-structural part of the virus is limited to that of JFH1. Hiraga *et al.*[14] have shown that infectious clones of genotype 1a and JFH1 can be infected with direct injection of *in vitro* transcribed RNA into the mouse liver.[14] Similarly, Kimura *et al.*[15] reported the establishment of infectious clones of genotype 1b and ablation of RNA polymerase by site-directed mutagenesis abolish infectivity. These infectious clones will be useful for the study of drug-resistant strains.

The model of HCV infection has also been used to show that infection of the virus can be prevented by antibodies against

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Table 1 New therapeutic strategies tested by human hepatocyte chimeric mice

n	Drug or cell	Strategy	Reference
1	Interferon alpha 2b	Activation of antiviral genes	Kneteman *et al.*[65]
	BILN-2061	NS3-4A protease inhibition	
	HCV371	NS5B polymerase inhibition	
2	Modified BID	Induction of apoptosis	Hsu *et al.*[66]
3	Serine palmitoyltransferase inhibitor	Disruption of lipid raft	Umehara *et al.*[67]
4	Lymphoblastoid interferon alpha	Activation of antiviral genes	Hiraga *et al.*[14]
5	Amphipathic DNA polymers	Blocking viral entry	Matsumura *et al.*[60]
6	Sec-butyl-analogue of HCV-371	NS5B polymerase inhibition	LaPorte *et al.*[68]
7	HCV796	NS5B polymerase inhibition	Kneteman *et al.*[69]
8	Liver allograft-derived lymphocyte	Adoptive immunotherapy	Ohira *et al.*[70]
9	Telaprevir	NS3-4A protease inhibition	Kamiya *et al.*[71]
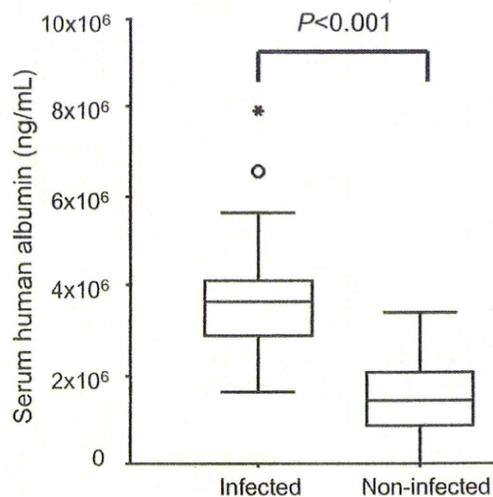
Figure 3 Human albumin levels in mice used in the hepatitis C virus (HCV) infection experiments. A total of 54 mice were injected with HCV positive serum samples containing 5×10^5 virus particles. A total of 24 mice became persistently positive for HCV-RNA, but 30 mice did not. Serum human albumin levels 2 weeks after human hepatocyte transplantation were compared between infected and non-infected mice.

CD81,[58] polyclonal human immunoglobulin directed to a similar strain,[59] and amphipathic DNA polymers.[60] Notably, the presence of broadly neutralizing antibodies to HCV that protect against heterologous viral infection has been reported, suggesting the possibility of a prophylactic vaccine against HCV.[61]

With respect to evasion of the virus against the innate immune response, altered intrahepatic expression profiles in the early phase of infection is of particular interest. The chimeric mice model is ideal for such studies; cross-hybridization of mouse and human can be avoided by careful experimental procedures.[62] Microarray analysis of livers of HCV infected and non-infected mice showed transcriptional activation of genes related to innate immune response, lipid metabolism, endoplasmic reticulum (ER) stress and apoptosis in HCV-infected mice.[63,64] The HCV infected mouse model is particularly useful for the study of newly developed HCV agents. The effect of recently developed chemicals and a unique therapy using intrahepatic lymphocytes have been shown using

this model (Table 1). However, none of these therapies have yet been able to completely eradicate HCV from mice. It is noteworthy that ultra-rapid cardiotoxicity has been reported with the protease inhibitor BILN 2061 in the uPA/scid mice, but not in scid mice, implicating involvement of the uPA transgene.[72] Care should therefore be taken in interpreting the results obtained by this model.

Conclusion

Development of a small animal model using human hepatocyte chimeric mice has enabled us to study key aspects of HBV and HCV biology. The characteristic feature of the absence of human immune cells is suitable for studying viral replication and observing changes occurring in liver cells during viral infection, such as the innate immune response and cellular stress and metabolic responses. The model is also useful for studying the effect of drugs without the influence of cytokines and cytotoxic T lymphocytes. Nonetheless, the model is insufficient to study carcinogenesis of hepatitis viruses, because non-parenchymal cells in mouse liver are of mouse origin and do not support inflammation and fibrosis, which are probably closely related to carcinogenesis. The lack of human immune cells also limits the study of inflammation and immunity. Furthermore, the availability of human hepatocytes is limited. Despite these limitations, the current model shows great potential as a mouse model for the study of hepatitis viruses. Development of a small animal model with or without human immunity using stem cells or iPS cells would be an ideal model in the future.

Acknowledgments

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