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Evaluation of antiviral effect of type I, II, and III interferons on direct-acting antiviral-resistant hepatitis C virus

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ABSTRACT

Treatment of hepatitis C virus (HCV) infection has greatly improved in the last 5 years because of the identification of direct-acting antivirals (DAAs). However, concerns exist regarding the emergence of drug resistance-associated substitutions (RASs). In this study, we evaluated the *in vivo* antiviral effect of three classes of interferons (IFNs), namely, types I, II, and III IFNs, on DAA-resistant HCVs. IFN-α_2, IFN-γ, and IFN-λ_1 were selected as typical types I, II, and III IFNs, respectively. Human hepatocyte-transplanted chimeric mice were infected with NS3-D168, NS5A-L31-, and NS5A-Y93-mutated HCVs, and the antiviral effect of IFN-α_2, IFN-γ, and IFN-λ_1 on these HCV RASs was examined. Chimeric mice infected with NS3- and NS5A-mutated HCVs were hydrodynamically injected with IFN-expressing plasmids to evaluate the antiviral effect of IFNs. Serum concentrations of IFNs were maintained for at least 42 days. We found that serum HCV level significantly decreased and serum and hepatic HCV levels reached below detection limit in 5/5 and 3/5 chimeric mice injected with IFN-γ- and IFN-λ_1-expressing plasmids, respectively. The antiviral effect of IFN-α_2 on DAA-resistant HCVs was weaker than that of IFN-γ and IFN-λ_1. Serum ALT levels showed a small and transient increase in mice injected with the IFN-γ-expressing plasmid but not in mice injected with the IFN-λ_1-expressing plasmid. However, no apparent histological damage was observed in the liver sections of mice injected with the IFN-γ-expressing plasmid. These results indicate that IFN-γ and IFN-λ_1 are an attractive therapeutic option for treating infection caused by NS3- and NS5A-mutated HCV.

1. Introduction

Hepatitis C virus (HCV) infection is a common cause of chronic hepatitis, cirrhosis, and hepatocellular carcinoma and affects more than 185 million people worldwide (Mohd et al., 2013). Conventional regimen for treating chronic HCV infection includes pegylated interferon-α (IFN-α) and ribavirin therapy, which shows limited therapeutic efficacy depending on HCV genotypes (Chayama et al., 2011; Puig-Basagoiti et al., 2005). In the previous decade, direct-acting antivirals (DAAs), which are synthetic inhibitors of HCV nonstructural (NS) proteins, were developed and approved for treating chronic HCV infection (Majumdar et al., 2016). A combination of first-generation DAAs such as telaprevir and simeprevir, NS3/4 protease inhibitors, with IFN-α and ribavirin exerts improved therapeutic effects in patients with chronic HCV infection. Moreover, treatment with next-generation DAAs such as ledipasvir and sofosbuvir, NS5A and NS5B inhibitor, respectively, allows IFN-free therapy. Results of clinical studies have reported high therapeutic efficiency of a combination ledipasvir tablet and sofosbuvir (Wyles et al., 2015; Kohli et al., 2015). In particular,

Abbreviations: HCV, hepatitis C virus; DAA, direct-acting antiviral; RASs, resistance-associated substitutions; IFN, interferon; NS, nonstructural; uPA/SCID, immunodeficient urokinase-type plasminogen activator; gLuc, *Gaussia* luciferase; fLuc, firefly luciferase; HSA, human serum albumin; ALT, alanine aminotransferase; PCR, polymerase chain reaction; ISG, IFN-stimulated gene; MxA, myxovirus-resistance protein A; PKR, RNA-dependent protein kinase; OAS, 2′,5′-oligoadenylate synthetase.

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sofosbuvir/velpatasvir/voxileprevir regimen showed the sustained virologic response (SVR) rates >95% except for genotype 1a HCV (Lawitz et al., 2016; Kevin et al., 2016). However, DAA therapy is associated with concerns such as appearance of drug resistance-associated substitutions (RASs) (Chayama and Hayes, 2015; Cento et al., 2015). Therefore, it is necessary to determine other regimens for treating infection caused by HCV RASs. According to the recent clinical study (Pawlotsky, 2016; Itakura et al., 2015; Zhang et al., 2016), re-treatment using DAA and IFN-based regimen was effective for the treatment of DAA failure patients. Although DAA therapy has been standard of care for HCV patients, IFN-based regimen has also contributed for the treatment of HCV for a long time. Therefore, we focused on IFN-based regimen as an optional regimen to treat DAA failure patients.

IFNs are a type of cytokines and are classified into three types, namely, type I IFNs such as IFN-α and IFN-β, type II IFNs such as IFN-γ, and type III IFNs such as IFN-λs (IFN-λ_1 [interleukin {IL}-29], IFN-λ_2 [IL-28A], and IFN-λ_3 [IL-28B]) (George et al., 2012). All IFN types exert antiviral, immunomodulatory, and anticancer effects. However, different IFNs have different receptors and signal transduction pathways (Sadler and Williams, 2008), which may affect their antiviral effect on HCV. Responsiveness of IFN to HCV has been evaluated. For instance, induction of IFN after infection of HCV (Qashqari et al., 2013; Oshiumi et al., 2013) and the detail of antiviral activity of type I IFN against HCV (Scagnolari and Antonelli, 2013) were investigated. However, information regarding the antiviral effect on DAA-resistant HCV was insufficient. Although previous studies have reported the antiviral effect of types I, II, and III IFNs on HCV (Tan et al., 2005; Panigrahi et al., 2013), their effect on infection caused by DAA RASs of HCV has not been reported to date.

In the present study, we determined IFN types for effectively treating infections caused by NS3- and NS5A-mutated HCVs. For this, human liver chimeric mice established by transplanting human hepatocytes into immunodeficient urokinase-type plasminogen activator (uPA/SCID) mice were infected with NS3-D168-, NS5A-L31-, and NS5A-Y93-mutated HCVs (Chayama et al., 2011; Takahashi et al., 2014). Previously, we showed that transfection of chimeric mice with a human IFN-γ-expressing plasmid provided sustained supply of IFN-γ in these mice and that the expressed IFN-γ exerted a strong antiviral effect against wild-type genotype 1b HCV (Takahashi et al., 2014). In the present study, we used human liver chimeric mice to evaluate whether sustained supply of IFN-α_2, IFN-γ, and IFN-λ_1, which are typical types I, II, and III IFNs, respectively, effectively treated infection caused by NS3- and NS5A-mutated HCVs.

2. Materials and methods

2.1. Plasmid DNA

Plasmids pCpG-IFN-γ and pCpG-gLuc expressing human IFN-γ and *Gaussia* luciferase (gLuc), respectively, were constructed as reported previously (Takahashi et al., 2014). Genes expressing human IFN-α_2 and IFN-λ_1 were synthesized by GenScript (Piscataway, NJ, USA) and FASMAC (Kanagawa, Japan), respectively. Plasmids pCpG-IFN-α_2 and pCpG-IFN-λ_1 were constructed by inserting IFN-α_2 and IFN-λ_1 gene fragments digested by *Kpn*I/*Nhe*I and *Bgl*II/*Nco*I, respectively, into *Kpn*I/*Nhe*I and *Bgl*II/*Nco*I sites, respectively, of plasmid pCpG-mcs (InvivoGen, San Diego, CA, USA). Plasmid pMx-IFN-λ_1 was constructed by replacing the enhancer and promoter regions of pCpG-IFN-λ_1 with the Mx promoter of pMx-mcs, as reported previously (Hamana et al., 2016).

2.2. Analysis of antiviral effect in vitro

LucNeo#2 cells, which are Huh-7 cells harboring self-replicating subgenomic HCV RNA replicons with a firefly luciferase (fLuc) reporter (Goto et al., 2006), were kindly provided by Dr. Hijikata and Dr. Shimotohno. LucNeo#2 cells were cultured, as reported previously (Uno et al., 2014). Briefly, LucNeo#2 cells were seeded (density, 4×10^4 cells/well) in 24-well culture plates 1 day before transfection. Next, the cells were cultured with 500 μL/well Opti-MEM (Thermo Fisher Scientific, Waltham, MA, USA) containing 5 μg of the indicated plasmid and 4 μL X-tremeGENE HP DNA Transfection Reagent (Roche Applied Science, Mannheim, Germany). At 1 and 3 days after the transfection, conditioned media were collected for determining IFN concentrations. Cell lysates were prepared using a lysis buffer provided in a luciferase assay kit (Piccagene Dual; Toyo Ink, Tokyo, Japan) and were mixed with fLuc assay kit (Piccagene, Toyo Ink). Chemiluminescence was measured using a luminometer (Lumat LB 9507; EG and G Berthold, Bad Wildbad, Germany).

2.3. Animal treatment

Immunodeficient uPA$^{+/+}$/SCID$^{+/+}$ mice were generated, and human hepatocytes were transplanted, as described previously (PhoenixBio Co., Ltd., Higashi-Hiroshima, Japan) (Tateno et al., 2004). All the mice were transplanted with frozen human hepatocytes obtained from the same donor. All animal protocols were performed in accordance with the guidelines of local committees for animal experiments (Hiroshima University and Kyoto University), and all the animals received humane care. Chimeric mice showing a high replacement rate for human hepatocytes were used in this study. Eight weeks after hepatocyte transplantation, the mice were intravenously injected with 10^5 copies of HCV. Human serum samples containing high titers of NS3-D168A/G-, NS5A-L31M-, and NS5A-Y93H-mutated genotype 1b HCVs were obtained from a patient with chronic hepatitis C who did not respond to daclatasvir plus asunaprevir treatment (Kan et al., 2016). The serum contained almost complete mutated HCV. The patient provided written informed consent for participating in the study. Study protocol conformed to the ethics guidelines mentioned in the 1975 Declaration of Helsinki and was approved by the review committee of the Hiroshima University.

2.4. Detection of drug-resistant substitutions

Amino acid sequences and population frequencies of NS3-D168, NS5A-L31, and NS5A-Y93-mutated HCV RASs were determined by performing Invader assay, as described previously (Yoshimi et al., 2015). Lower detectable limit for population frequency in the Invader assay was set as 1%.

2.5. In vivo gene transfer

The chimeric mice were hydrodynamically injected with 250 μg plasmid (Liu et al., 1999). Briefly, plasmids dissolved in saline (volume, 10% of body weight) were injected into the tail vein of the chimeric mice within 5 s.

2.6. Measurement of IFN concentrations, gLuc activity, and human serum albumin and alanine aminotransferase levels

Serum samples were collected from the mice at indicated times after the plasmid injection and were stored at −80 °C until further analysis. IFN concentrations in the conditioned media of plasmid-transfected LucNeo#2 cells and those in the serum samples of