Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochemical and Biophysical Research Communications 346 (2006) 67–73
BBRC
www.elsevier.com/locate/ybbrc

Serine palmitoyltransferase inhibitor suppresses HCV replication in a mouse model

Takuya Umehara [a], Masayuki Sudoh [b], Fumihiko Yasui [a], Chiho Matsuda [a], Yukiko Hayashi [c], Kazuaki Chayama [d], Michinori Kohara [a,*]

[a] *Department of Microbiology and Cell Biology, The Tokyo Metropolitan Institute of Medical Science, 3-18-22 Honkomagome, Bunkyo-ku, Tokyo 113-8613, Japan*
[b] *Kamakura Research Laboratories, Chugai Pharmaceutical Co., Ltd., 200 Kajiwara, Kamakura, Kanagawa 247-8530, Japan*
[c] *Department of Pathology, Tokyo Metropolitan Komagome Hospital, 3-18-22 Honkomagome, Bunkyo-ku, Tokyo 113-8677, Japan*
[d] *Department of Medicine and Molecular Science, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Sciences, Hiroshima University, Minami-ku, Hiroshima-shi, 734-8551, Japan*

Received 26 April 2006
Available online 24 May 2006

Abstract

Serine palmitoyltransferase (SPT) is a first-step enzyme in the sphingolipid biosynthetic pathway. Myriocin is an inhibitor of SPT and suppresses replication of the hepatitis C virus (HCV) replicon. However, it is still unknown whether this SPT inhibitor suppresses HCV replication in vivo. We investigated the anti-HCV effect of myriocin against intact HCV using chimeric mice with humanized liver infected with HCV genotype 1a or 1b. We administered myriocin into HCV infected chimeric mice and succeeded in reducing the HCV RNA levels in serum and liver to 1/10–1/100 of the levels prior to the 8 day treatment. Furthermore, combined treatment with pegylated interferon reduced the HCV RNA levels to less than 1/1000 of the control levels. We strongly suggest that suppression of SPT reduces HCV replication, and therefore that the SPT inhibitor is potentially a novel drug in the treatment of HCV infection.

Keywords: HCV; Myriocin; Serine palmitoyltransferase; Lipid raft; Chimeric mice with humanized liver

Hepatitis C virus (HCV) infection usually causes chronic hepatitis and often leads to cirrhosis of the liver or hepatocellular carcinoma [1,2]. The number of carriers now amounts to approximately 3% (~170 million) of the population worldwide. The most effective treatment against HCV infection is a combination of pegylated interferon (PEG-IFN) and ribavirin [3,4]. However, many people cannot tolerate the serious side effects and thus the number of patients able to receive the therapy is limited. The development of novel drugs to treat HCV with greater safety and better efficacy is therefore urgently required.

HCV is a single-stranded RNA virus that belongs to the Flaviviridae family [5]. The RNA genome produces at least 10 viral proteins, which include structural and non-structural (NS) proteins. The former are involved in the formation of the HCV particle. The latter play a key role in HCV genome replication [6]. It is generally accepted that a complex of NS proteins is associated with the lipid raft on the Golgi and endoplasmic reticulum membranes, where HCV replication occurs [7,8]. Thus, disruption of assembly of the lipid raft may lead to suppression of HCV replication.

Myriocin (ISP-1) is a specific inhibitor of serine palmitoyltransferase (SPT), a first-step enzyme in the sphingolipid biosynthetic pathway (Fig. 1A; [9,10]). Myriocin inhibits SPT activity due to its structural similarity to sphingosine (Fig. 1B), resulting in decreased intercellular sphingomyelin and its intermediates, dihydrosphingosine, sphingosine, ceramide, and sphingosine-1-phosphate (Fig. 1A). Inhibition of SPT by myriocin is thought to eventually lead to disruption of lipid raft assembly, as sphingomyelin is one of the major integral components of its assembly [11].

* Corresponding author. Fax: +81 3 3828 8945.
E-mail address: mkohara@rinshoken.or.jp (M. Kohara).

0006-291X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2006.05.085

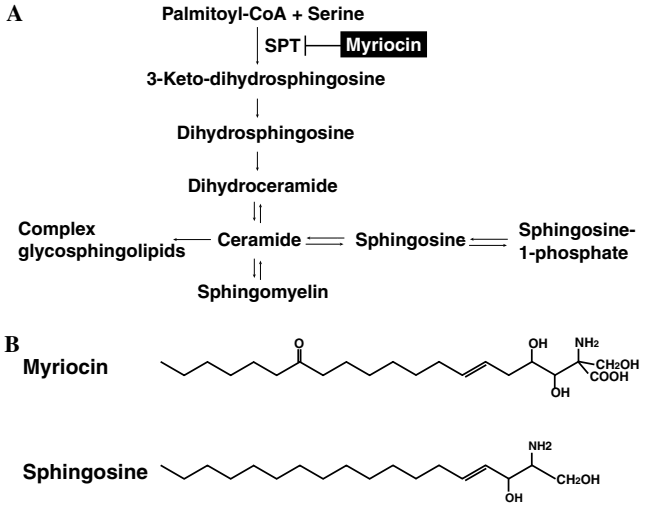

Fig. 1. Sphingolipid biosynthetic pathway. (A) Biosynthesis pathway of sphingolipid. (B) Structure of myriocin and sphingosine.

Previously, we isolated the compound NA255, which suppresses replication of the HCV subgenomic replicon [12]. NA255 is structurally similar to myriocin and inhibits the enzymatic activity of SPT, resulting in suppression of replication without affecting the enzyme activities of HCV NS3 (protease and helicase) or NS5B (RNA-dependent RNA polymerase). Thus, replication of the HCV subgenomic replicon was suppressed by NA255 in response to the decrease in amount of sphingolipid, ceramide, and sphingomyelin. These findings suggest that NA255 disrupts assembly of the lipid raft associated with HCV NS proteins. In the present study, we performed comparative and concomitant trials of one SPT inhibitor, myriocin, and PEG-IFN in chimeric mice with humanized liver (chimeric mice) infected with intact HCV. The results demonstrate for the first time that suppression of SPT inhibits replication of intact HCV in vivo.

Materials and methods

Inhibition assay of replication in HCV replicon cells by myriocin. Myriocin (Sigma, St. Louis, MO, USA) was added in the growth medium of HCV subgenomic replicon cells FLR3-1 (genotype 1b, Con-1; [12]) at a final concentration of 0.2, 1.0, 3.9, 15.6 or 62.5 nM. After 72 h incubation, we performed luciferase assays using the Bright-Glo luciferase assay kit (Promega, Madison, WI, USA).

Measurement of cell viability using the Tetra Color One (WST-8) assay. Myriocin was added to FLR3-1 cells as described above. After 72 h incubation, cell viability was measured using the Tetra Color One kit (Seikagakukougyo, Tokyo, Japan) according to the manufacturer's instructions.

Immunoblotting analysis. Cells were harvested and lysed in lysis buffer (PBS containing 0.5% Triton X-100 and 0.5 mM PMSF), and then 5 μg of protein separated by 12% SDS–PAGE and electro-blotted onto a nitrocellulose membrane (Shleicher & Schuell, Dassel, Germany). A rabbit polyclonal anti-NS3 antibody [12] and anti-actin (20–33) antibody (Sigma, St. Louis, MO, USA) were used as the primary antibodies. The proteins were then detected by an anti-rabbit antibody HRP-linked IgG (Cell Signaling Technology, Beverly, MA, USA).

Immunofluorescent staining of HCV replicon cells. After treatment of 250 nM myriocin for 72 h, FLR3-1 cells were probed with a primary antibody, an anti-NS3 polyclonal antibody, after blocking with TNB blocking buffer (Perkin-Elmer, Wellesley, MA, USA). Next, an anti-rabbit IgG-Alexa-488 conjugate (Invitrogen, Carlsbad, CA, USA) was applied as the secondary antibody.

TLC analysis. Cells were incubated for 2 h with [^{14}C] serine (0.5 μCi/ml) in Opti-MEM (Invitrogen). After the cells were lysed with 0.1% SDS, and total lipids were extracted with chloroform/methanol (1:2 v/v). The extracts were spotted onto Silica Gel 60 thin-layer chromatography (TLC) plates (Merck, Darmstadt, Germany) and chromatographed with methyl acetate/1-propanol/chloroform/methanol/0.25% KCl (25:25:25:10:9, v/v). Radioactive spots were detected by BAS 2000 (Fuji Film, Kanagawa, Japan).

Complementation of sphingolipid intermediates. FLR3-1 cells were incubated with 1 or 2.5 μM of sphingolipid intermediates (dihydrosphingosine, sphingosine, or sphingosine-1-phosphate) and sequentially diluted myriocin then added. After 72 h, the IC_{50} of each combination was measured by the luciferase assay.

Infection of HCV genotype 1a and 1b in chimeric mice. Chimeric mice were purchased from PhenixBio Co., Ltd. (Hiroshima, Japan). The mice were generated by transplanting human primary hepatocytes into SCID mice carrying the urokinase plasminogen activator transgene controlled by an albumin promoter (*Alb-uPA*) [13–15]. Overexpression of this transgene induces a profoundly hypofibrinogenemic state and accelerated hepatocyte death. HCG9 (genotype 1a) and HCR6 (genotype 1b, Accession No: AY045702), originally from patient serum, were intravenously injected at 10^6 copies/mouse at about 40 days after transplantation of human hepatocytes. After 4 weeks, the HCV 1a and 1b RNA levels had reached $\sim 10^8$ copies/ml and $\sim 10^7$ copies/ml, respectively, in the mice serum.

Administration of myriocin and/or PEG-IFN into chimeric mice infected with HCV 1b. Injections of myriocin or PEG-IFN (Chugai, Tokyo, Japan) or both were administered to HCV genotype 1b (HCR6) infected mice and blood then collected according to the protocol in Table 2.

Quantification of HCV RNA by real-time PCR. Total RNA was purified from 1 μl of serum or 50 μg of liver tissue from chimeric mice using the AGPC method. HCV RNA was quantified by real-time PCR as previously reported [16].

Measurement of human albumin in the serum. Human albumin concentration was measured in 2 μl of serum using the Alb-II kit (Eiken Chemical, Tokyo, Japan) according to the manufacturer's instructions.

Detection of core protein in live tissue. We used chimeric mice with a high RNA levels of HCV genotype 1a (HCG9) in the serum to easily detect HCV RNA and core protein in liver tissue. We administered 2 mg/kg myriocin daily for 6 days to a chimeric 1a-4 mouse and extirpated the liver. For comparison, the livers of non-treated (1a-1), non-infected (1a-2), and PEG-IFN treated (1a-3) mice were also extirpated. The liver tissues were homogenized in RIPA buffer and 100 μg of total protein was used for the detection of core protein using the Ortho HCV core protein ELISA kit (Eiken Chemical).

Immunofluorescent and histological staining of chimeric mouse liver tissue. Liver sections from 1a-1 and 1a-4 mice were probed by biotinylated anti-HCV core protein monoclonal antibody, and human hepatocyte monoclonal antibody (Dako, Glostrup, Denmark) as the primary antibodies, followed by streptavidin-Alexa-488 (Invitrogen) and anti-mouse-IgG-Alexa-546 (Invitrogen). The nuclei were stained using DAPI. Biotinylated normal mouse IgG (Ancell, Bayport, MN, USA) was used as the negative control. For histological analysis, liver sections from 1a-1 and 1a-4 mice were stained by hematoxylin-eosin (H&E staining).

Results

Anti-HCV effect of the SPT inhibitor, myriocin

We examined the anti-HCV effect and cell toxicity of myriocin in the HCV subgenomic replicon cells FLR3-1. Luciferase activity was greatly decreased by myriocin in a dose-dependent manner without affecting cell viability

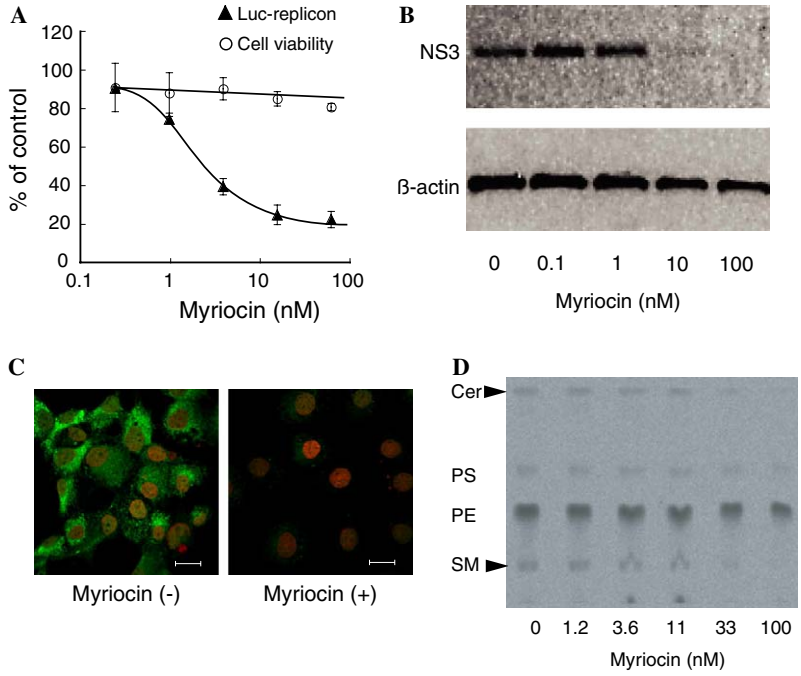

Fig. 2. Anti-HCV effect of myriocin in HCV replicon cells. (A) Luciferase activity and cell viability of FLR3-1 replicon cells in the presence of myriocin. Experiments were conducted independently at least three times. (B) Detection of NS3 protein by immnoblotting analysis. (C) Detection of NS3 protein by immunofluorescent staining in FLR3-1 cells. HCV NS3 protein and nucleus are shown in green and red, respectively. Scale bar: 20 μm. (D) De novo sphingolipid biosynthesis in the presence of myriocin was monitored by TLC. Cer, ceramide; PS, phosphatidylserine; PE, phosphatidylethanolamine; and SM, sphingomyelin.

Table 1
IC_{50} values of myriocin in the presence of sphingolipids

Supplement (μM)		IC_{50} of myriocin (nM)
Absence	0	5.8
Dihydrosphingosine	1.0	77.7
	2.5	>1000
Sphingosine	1.0	22.4
	2.5	>1000
Sphingosine-1-phosphate	1.0	14.7
	2.5	>1000

IC_{50} values of myriocin measured in the presence of 1, and 2.5 μM dihydrosphingosine, sphingosine or sphingosine-1-phosphate, show suppression of HCV replicon replication by myriocin.

(Fig. 2A) or cell growth (data not shown). The maximum inhibition rate was about 80% in the presence of over 62.5 nM myriocin (Fig. 2A), while the 50% inhibitory concentration (IC_{50}) was about 5.8 nM (Table 1). Reduction of NS3 protein, which plays a key role in HCV replication, was also observed by immunoblotting analysis and staining (Figs. 2B and C), suggesting that myriocin has a potent anti-HCV effect.

Relationship between sphingolipid metabolites and HCV replication

To examine the relationship between sphingolipid metabolites and HCV replication, we monitored de novo sphingolipid biosynthesis by FLR3-1 cells in the presence of myriocin. The production of both ceramide and sphingomyelin was inhibited in a dose-dependent manner, whereas production of the phosphatidylethanolamine and phosphatidylserine, metabolites of sphingosine, was unaffected (Fig. 2D). To confirm whether suppression of HCV subgenomic replicon replication was caused by sphingolipid depletion, we examined the anti-HCV effect of myriocin in the presence of three sphingolipids, dihydrosphingosine, sphingosine, and sphingosine-1-phosphate, which are intermediates in the sphingolipid biosynthesis pathway (Fig. 1A). Replication of the HCV replicon was recovered by complementation of the intermediate molecules of sphingolipid biosynthesis (Table 1). These results indicate that suppression of replication by myriocin was due to a reduction in sphingolipid biosynthesis.

Anti-HCV effects of myriocin and PEG-IFN in chimeric mice infected with HCV

The inhibitory ability of myriocin was investigated using chimeric mice infected with HCR6 (genotype 1b). We administered myriocin or PEG-IFN via intraperitoneal or subcutaneous injection, as shown in Table 2. In the myriocin-treated group, the HCV RNA levels were reduced in the serum from 3×10^6–1×10^7 copies/ml to 6×10^5–1×10^4 copies/ml over 8 days (an approximately 1/10–1/100 reduction). The same level of reduction was

Table 2
Administration schedule into chimeric mice infected with HCV genotype 1b

Day	−1	0	1	2	3	4	5	6	7	8
Collection of blood	B		B			B				B
PEG-IFN		I			I				I	
Myriocin		M	M	M	M	M	$M_{1/2}$	$M_{1/2}$	$M_{1/2}$	
Myriocin + PEG-IFN		M/I	M	M	M/I	$M_{1/2}$	$M_{1/2}$		I	

B, I or M indicates that each manipulation was performed as required, and administration of reagents was started from day zero. PEG-IFN was subcutaneously injected at 30 μg/kg. The amount of myriocin intraperitoneally injected was adjusted according to the body weight of the mice. Doses began at 1 mg/kg, and at a 10% reduction in body weight, the dose was reduced to 0.5 mg/kg ($M_{1/2}$). At 20% reduction, administration was discontinued.

observed in the PEG-IFN-treated group, which was injected with a 10-fold larger amount of PEG-IFN than that used in clinical treatment (30 μg/kg body weight). Moreover, combined treatment with myriocin and PEG-IFN reduced the HCV RNA levels to less than 1/1000 of the control levels, and in 2 (1b-7 and 1b-9) of 3 mice HCV RNA was not detected at all on day 8 (Fig. 3A). Concurrently, we monitored the concentration of human albumin (h-Alb) and found slight reductions only in the combined treatment group (Fig. 3B). These results indicate that myriocin suppressed replication of intact HCV without interfering with h-Alb expressed from humanized liver, whereas the combination of myriocin and PEG-IFN synergistically suppressed HCV replication with slight liver damage.

Detection of HCV RNA and core protein in the liver of chimeric mice

To clarify whether HCV was reduced by myriocin from the humanized liver, we examined the livers of the chimeric mice infected with another HCV genotype, 1a (HCG9). The RNA level of HCV 1a in serum reached approximately 1×10^8 copies/ml, which is 10-fold higher than that of HCV 1b (HCR6). Thus, we speculated that HCV core protein in hepatocytes would be easily detected by immunofluorescent staining. The liver of a 1a-4 mouse was extirpated after daily administration of 2 mg/kg of myriocin for 6 days. Following treatment, the HCV RNA level in the serum of the 1a-4 mouse fell to 1×10^5 copies/ml (Fig. 4A). The amount of HCV 1a RNA and core protein

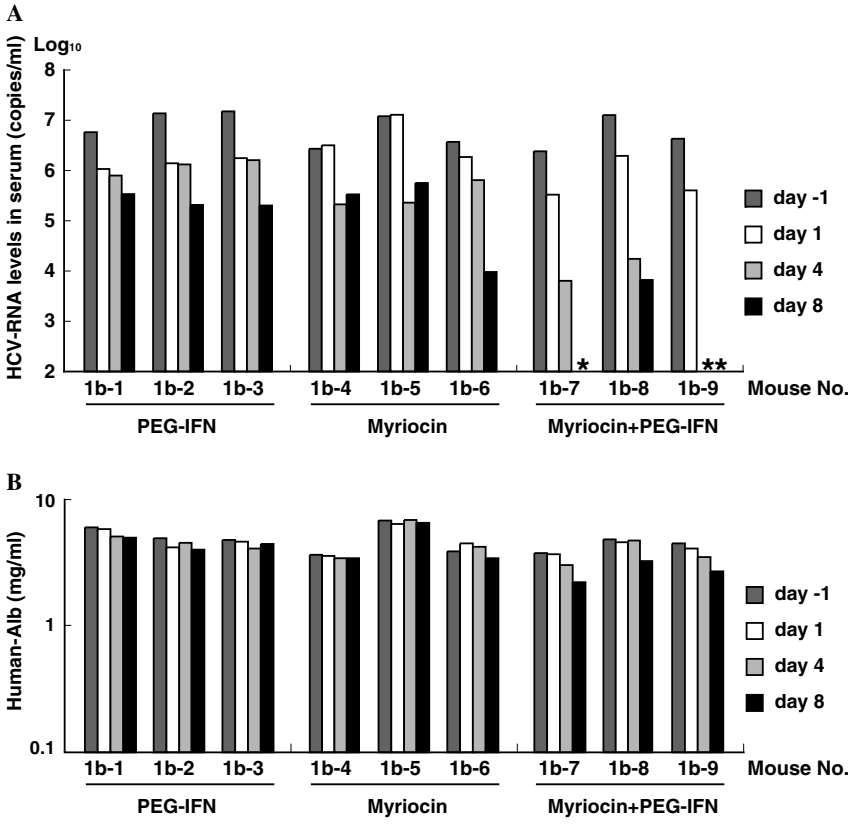

Fig. 3. Anti-HCV effect of myriocin in chimeric mice infected with HCV genotype 1b. (A) HCV RNA levels in the serum of chimeric mice. Asterisks indicate no HCV RNA was detected. (B) Human albumin concentrations in serum of chimeric mice.

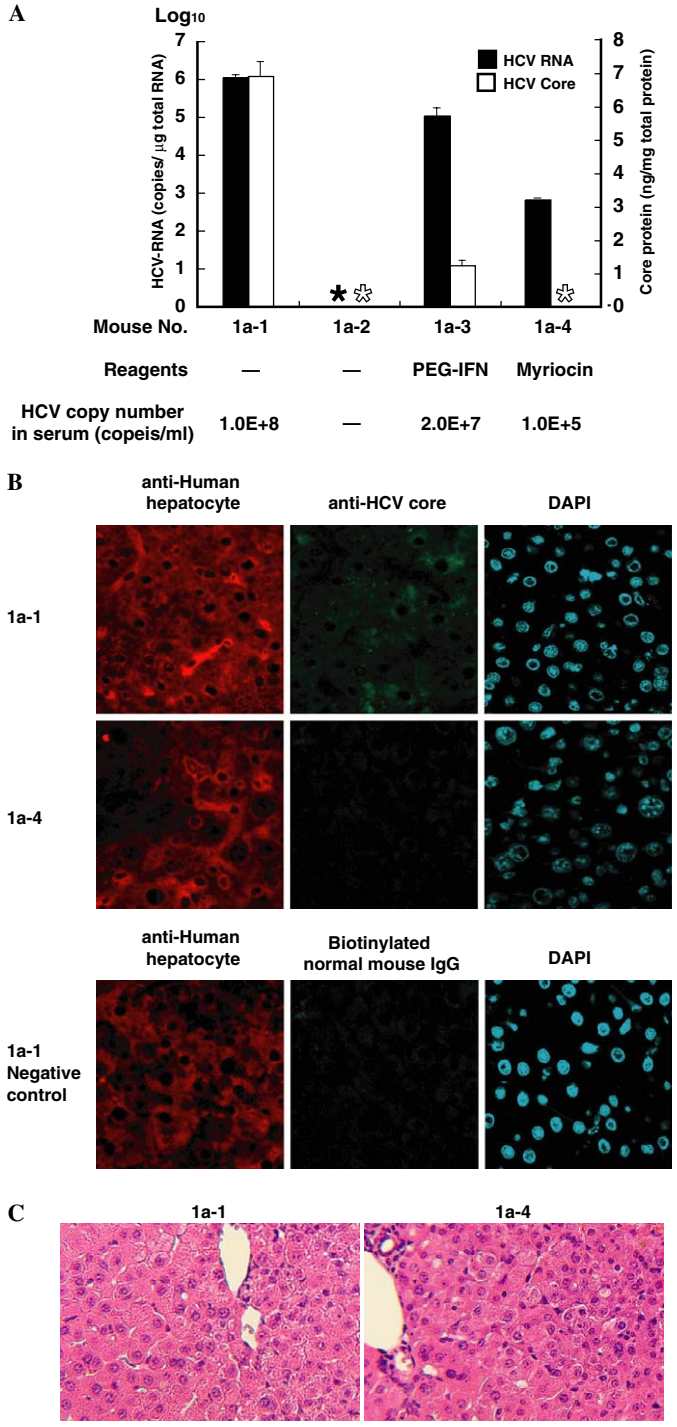

Fig. 4. Analysis of liver tissue from chimeric mice. HCV RNA and core protein were detected in the liver of chimeric mice infected with HCV genotype 1a. 1a-1, non-treated; 1a-2, non-treated and non-infected; 1a-3, PEG-IFN treated; and 1a-4, myriocin treated mouse. (A) Copy number of HCV RNA per 1 μg total RNA, and expression levels of HCV core proteins per 1 mg total protein. Asterisks indicate no HCV RNA or core proteins were detected. (B) Immunofluorescent staining of HCV core protein and human hepatocytes in chimeric mice liver. Human hepatocytes, HCV core protein, and nucleus are shown in red, green, and light blue, respectively. As a negative control of anti-core monoclonal antibody staining, the liver of 1a-1 was also stained with biotinylated normal mouse IgG. (C) H&E staining of liver tissue from chimeric mice 1a-1 and 1a-4. Primary human hepatocytes were observed in these mice and these hepatocytes displayed no significant morphological changes.

in the liver was quantified and had also reduced, as well as that in serum (Fig. 4A). Immunofluorescent staining revealed that the core protein of the non-treated mouse, 1a-1, which had 1×10^8 copies/ml serum (Fig. 4A), expressed a human hepatocyte moiety, whereas the core protein of the 1a-4 mouse disappeared (Fig. 4B). These results indicate that myriocin causes a decrease not only in HCV genotype 1b but also in genotype 1a, and eliminates HCV from the liver.

H&E staining of chimeric mouse liver

We performed histological analysis of the livers from non-treated (1a-1) and myriocin-treated (1a-4) mice (Fig. 4C). No significant morphological differences were observed between the tissues of the 1a-1 and 1a-4 mice. Thus, myriocin did not induce hepatocyte damage in chimeric mice to any biologically significant degree.

Discussion

In the present study, the SPT inhibitor myriocin was shown to inhibit replication of intact HCV in vivo. We initially investigated the fundamental inhibitory effects and mechanisms of myriocin against replication of the HCV replicon and found that inhibition of HCV replicon replication is compatible with a decrease in ceramide and sphingomyelin in the cells. The inhibitory effect of myriocin on replication of the HCV replicon differed slightly from that on de novo biosynthesis of ceramide and sphingomyelin. It has been previously reported that the membranous web formed in HCV replicon cells is the site of viral RNA synthesis and is not observed in naive HuH-7 cells [17]. Furthermore, HCV replication is known to occur on the lipid raft assembly [7]. The lipid raft associated with HCV replicase exists as an aberrant structure that forms a membranous web, and thus the lipid raft formed by HCV differs from the one in healthy cells. Therefore, we speculate that the above difference indicates that the lipid raft associated with HCV replicase is sensitively disrupted by myriocin.

Furthermore, HCV replication inhibition was complemented in the presence of the sphingomyelin biosynthetic intermediates dihydrosphingosine, sphingosine, and sphingosine-1-phosphate. These results indicate that depletion of sphingolipids induces disruption of the lipid raft assembly, resulting in suppression of replication of the HCV replicon. Disruption of the lipid raft assembly would lead to failure of HCV replicase to associate with the raft, and thus interrupt genome replication. This result is consistent with a previous study [12]. In order to demonstrate whether inhibition of SPT can be linked to anti-HCV therapy in vivo, we used a chimeric mouse model.

Recently, Mercer et al. developed a chimeric mouse containing human hepatocytes in which infection and replication of intact HCV occurs [14]. We examined the anti-HCV effect of myriocin in chimeric mice infected with HCV

genotypes 1a and 1b, and succeeded in the effective elimination of both HCV genotypes in their serum. Myriocin did not induce significant liver damage or interfere with the amount of human albumin in the chimeric mice or the ALT levels in Balb/c mice (data not shown), and thus the reduction in HCV RNA levels by myriocin is not due to induction of human hepatocyte damage. In the analysis of liver tissue infected with HCV genotype 1a, both HCV RNA and core protein levels were reduced by myriocin more effectively than by PEG-IFN. Thus, myriocin appears to be able to directly suppress replication of intact HCV in human hepatocytes, regardless of the HCV genotype. This is the first report of an SPT inhibitor suppressing intact HCV replication in vivo. Furthermore, combined treatment was more effective than myriocin or PEG-IFN alone, with HCV RNA levels reduced to less than 1/1000 of the controls, suggesting that myriocin with PEG-IFN cooperatively and synergistically inhibits the replication and proliferation of HCV.

Cholesterol is another major component of the lipid raft assembly, in addition to the sphingolipids [11]. The cholesterol biosynthetic pathway has also been a target for disruption of lipid raft assembly. However, recent studies have shown that the protein associated with geranlygeranylation, rather than cholesterol, is important for HCV replication [7,18,19]. Thus, it appears likely that disruption of lipid raft assembly is effectively caused by inhibition of sphingolipid biosynthesis rather than inhibition of cholesterol biosynthesis.

Myriocin is a known immunosuppressant [9], and mainly inhibits generation of cytotoxic T lymphocytes and T-cell dependent antibody production via inhibition of SPT activity in vivo. In chimeric mice deficient in both T and B cells (SCID), the immunosuppressant effect of myriocin does not cause a reduction in HCV replication but simply causes disruption of sphingolipid biosynthesis. In addition, the inhibitory mechanism in vivo, as in vitro analysis has shown, is likely to be disrupted by myriocin of the lipid raft assembly. Whether the sphingolipid level in liver in vivo is disrupted is currently unknown and the focus of future study. We continually monitored HCV for 14 days after the administration regimen and detected the same level of HCV as before the administration among all groups. Thus, to eliminate HCV completely, it will be necessary to adjust the dosage of myriocin and PEG-IFN and further extend the duration of administration.

In conclusion, we elucidated the mechanism of myriocin inhibition of HCV replication in vitro and determined that myriocin inhibits HCV replication in a chimeric mouse model with humanized liver. Although the toxicity of myriocin renders it unsuitable for use as an anti-HCV drug in human patients, our results suggest that SPT may be an effective target of drugs designed to inhibit HCV replication, and that SPT inhibitors such as myriocin are good candidates on which to base the development of new anti-HCV drugs.

Acknowledgments

The authors thank PhenixBio Co., Ltd. for maintenance of the humanized-liver mice. We thank for I. Kusanagi for technical assistance and F. Ford for editorial assistance. This study was supported by grants from the Ministry of Education, Culture, Sports, Science, and Technology of Japan; the Program for Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation of Japan; and the Ministry of Health, Labor and Welfare of Japan.

References

[1] K. Kiyosawa, T. Sodeyama, E. Tanaka, Y. Gibo, K. Yoshizawa, Y. Nakano, S. Furuta, Y. Akahane, K. Nishioka, R.H. Purcell, et al., Interrelationship of blood transfusion, non-A, non-B hepatitis and hepatocellular carcinoma: analysis by detection of antibody to hepatitis C virus, Hepatology 12 (1990) 671–675.

[2] I. Saito, T. Miyamura, A. Ohbayashi, H. Harada, T. Katayama, S. Kikuchi, Y. Watanabe, S. Koi, M. Onji, Y. Ohta, et al., Hepatitis C virus infection is associated with the development of hepatocellular carcinoma, Proc. Natl. Acad. Sci. USA 87 (1990) 6547–6549.

[3] P. Glue, R. Rouzier-Panis, C. Raffanel, R. Sabo, S.K. Gupta, M. Salfi, S. Jacobs, R.P. Clement, A dose-ranging study of pegylated interferon alfa-2b and ribavirin in chronic hepatitis C. The Hepatitis C Intervention Therapy Group, Hepatology 32 (2000) 647–653.

[4] K.R. Reddy, T.L. Wright, P.J. Pockros, M. Shiffman, G. Everson, R. Reindollar, M.W. Fried, P.P. Purdum 3rd, D. Jensen, C. Smith, W.M. Lee, T.D. Boyer, A. Lin, S. Pedder, J. DePamphilis, Efficacy and safety of pegylated (40-kd) interferon alpha-2a compared with interferon alpha-2a in noncirrhotic patients with chronic hepatitis C, Hepatology 33 (2001) 433–438.

[5] Q.L. Choo, K.H. Richman, J.H. Han, K. Berger, C. Lee, C. Dong, C. Gallegos, D. Coit, R. Medina-Selby, P.J. Barr, et al., Genetic organization and diversity of the hepatitis C virus, Proc. Natl. Acad. Sci. USA 88 (1991) 2451–2455.

[6] S. Rosenberg, Recent advances in the molecular biology of hepatitis C virus, J. Mol. Biol. 313 (2001) 451–464.

[7] H. Aizaki, K.J. Lee, V.M. Sung, H. Ishiko, M.M. Lai, Characterization of the hepatitis C virus RNA replication complex associated with lipid rafts, Virology 324 (2004) 450–461.

[8] L. Gao, H. Aizaki, J.W. He, M.M. Lai, Interactions between viral nonstructural proteins and host protein hVAP-33 mediate the formation of hepatitis C virus RNA replication complex on lipid raft, J. Virol. 78 (2004) 3480–3488.

[9] T. Fujita, K. Inoue, S. Yamamoto, T. Ikumoto, S. Sasaki, R. Toyama, K. Chiba, Y. Hoshino, T. Okumoto, Fungal metabolites. Part 11. A potent immunosuppressive activity found in Isaria sinclairii metabolite, J. Antibiot. (Tokyo) 47 (1994) 208–215.

[10] Y. Miyake, Y. Kozutsumi, S. Nakamura, T. Fujita, T. Kawasaki, Serine palmitoyltransferase is the primary target of a sphingosine-like immunosuppressant, ISP-1/myriocin, Biochem. Biophys. Res. Commun. 211 (1995) 396–403.

[11] K. Simons, E. Ikonen, Functional rafts in cell membranes, Nature 387 (1997) 569–572.

[12] H. Sakamoto, K. Okamoto, M. Aoki, H. Kato, A. Katsume, A. Ohta, T. Tsukuda, N. Shimma, Y. Aoki, M. Arisawa, M. Kohara, M. Sudoh, Host sphingolipid biosynthesis as a target for hepatitis C virus therapy, Nat. Chem. Biol. 1 (2005) 333–337.

[13] K. Emoto, C. Tateno, H. Hino, H. Amano, Y. Imaoka, K. Asahina, T. Asahara, K. Yoshizato, Efficient in vivo xenogeneic retroviral vector-mediated gene transduction into human hepatocytes, Hum. Gene Ther. 16 (2005) 1168–1174.

[14] D.F. Mercer, D.E. Schiller, J.F. Elliott, D.N. Douglas, C. Hao, A. Rinfret, W.R. Addison, K.P. Fischer, T.A. Churchill, J.R. Lakey, D.L. Tyrrell, N.M. Kneteman, Hepatitis C virus replication in mice with chimeric human livers, Nat. Med. 7 (2001) 927–933.

[15] C. Tateno, Y. Yoshizane, N. Saito, M. Kataoka, R. Utoh, C. Yamasaki, A. Tachibana, Y. Soeno, K. Asahina, H. Hino, T. Asahara, T. Yokoi, T. Furukawa, K. Yoshizato, Near completely humanized liver in mice shows human-type metabolic responses to drugs, Am. J. Pathol. 165 (2004) 901–912.

[16] T. Takeuchi, A. Katsume, T. Tanaka, A. Abe, K. Inoue, K. Tsukiyama-Kohara, R. Kawaguchi, S. Tanaka, M. Kohara, Real-time detection system for quantification of hepatitis C virus genome, Gastroenterology 116 (1999) 636–642.

[17] R. Gosert, D. Egger, V. Lohmann, R. Bartenschlager, H.E. Blum, K. Bienz, D. Moradpour, Identification of the hepatitis C virus RNA replication complex in Huh-7 cells harboring subgenomic replicons, J. Virol. 77 (2003) 5487–5492.

[18] S.B. Kapadia, F.V. Chisari, Hepatitis C virus RNA replication is regulated by host geranylgeranylation and fatty acids, Proc. Natl. Acad. Sci. USA 102 (2005) 2561–2566.

[19] S.T. Shi, K.J. Lee, H. Aizaki, S.B. Hwang, M.M. Lai, Hepatitis C virus RNA replication occurs on a detergent-resistant membrane that cofractionates with caveolin-2, J. Virol. 77 (2003) 4160–4168.