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AASLD 2008 Annual Meeting

October 31 - 04, 2008

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Friday, October 31, 2008

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Saturday, November 01, 2008

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Sunday, November 02, 2008

Time	Session Info
8:00 AM-5:30 PM, West Hall (Moscone West Convention Center), **HBV: Pathogenesis**	
8:00 AM-5:30 PM	**817. HBx protein is necessary for HBV replication in human hepatocytes.** M. Tsuge; N. Hiraga; M. Imamura; S. Takahashi; K. Chayama
8:00-8:00 AM	**818. Reactive oxygen species induce liver fibrosis in uPA/SCID mice with human hepatocytes infected with specific hepatitis B virus genotypes** M. Sugiyama; Y. Tanaka; I. Maruyama; T. Shimada; S. Takahashi; T. Shirai; K. Hino; I. Sakaida; M. Mizokami
8:00 AM-5:30 PM, West Hall (Moscone West Convention Center), **HBV: Treatment and Clinical Trials**	
8:00 AM-5:30 PM	**949. Serine palmitoyltransferase inhibitor suppresses hepatitis B virus replication** K. Tatematsu; Y. Tanaka; M. Sugiyama; M. Mizokami

Monday, November 03, 2008

Time	Session Info
8:00 AM-5:30 PM, West Hall (Moscone West Convention Center), **HCV: Therapy**	
8:00 AM-5:30 PM	**1226. Evaluation of Pegylated Interferon plus Ribavirin-induced Selection and prevalence of Amino acid 70Q in Core of HCV-1b** F. Kurbanov; Y. Tanaka; K. Matsuura; F. Sugauchi; T. Sakamoto; I. Hasegawa; T. Ohno; M. Mizokami

Tuesday, November 04, 2008

Time	Session Info
8:00 AM-12:30 PM, West Hall (Moscone West Convention Center), **HCV: Preclinical and Clinical Trials**	

8:00 AM-12:30 PM	**1906. Telaprevir treatment in combination with interferon-alpha to a human hepatocyte chimeric mouse with hepatitis C virus infection** E. Iwao; M. Imamura; N. Hiraga; T. Kimura; D. Miki; T. Hatakeyama; F. Mitsui; M. Tsuge; N. Kamiya; S. Takahashi; K. Chayama

Final ID: 817

HBx protein is necessary for HBV replication in human hepatocytes.

M. Tsuge; [1]; *N. Hiraga;* [1]; *M. Imamura;* [1]; *S. Takahashi;* [1]; *K. Chayama;* [1];
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Abstract Body: Background & Aims: The HBx protein is one of the hepatitis B virus (HBV) proteins that is considered to play an important role in replication of the hepatitis B virus. Previous reports have shown that the X protein is important in the replication of the woodchuck hepatitis virus, but not in the duck hepatitis B virus. Recently, we developed reverse genetics of the HBV using human hepatocyte chimeric mice. In this study, we examined whether or not the X protein of the HBV is dispensable in viral replication in vivo.

Methods: For expression of the HBx protein, we constructed plasmids containing the entire HBx protein in pcDNA3 and pcDNA3-HA plasmid vectors. A stop codon was introduced to the HBx region of the plasmid containing the entire HBV genome with C1395T nucleotide substitution (CAA to TAA). Wild and HBx-deficient HBV particles were produced from HepG2 cells after transfection of 1.4 genome length plasmids with the calcium phosphate method. Produced HBV particles were inoculated into the human hepatocyte chimeric mice from the tail vein, with or without a hydrodynamic injection of HBx expression plasmids. We extracted the serum samples from the mice every two weeks and analyzed the HBV titers by real time PCR.

Results: The HBx protein expression by hydrodynamic injection of the HBx protein expressing plasmid was confirmed by the Western Blot analysis, using antibody directed to HA tag. Quantitatively measurable viremia developed in six of seven HBx injected mice. In contrast, none of the 16 mice without the expression of the HBx developed the measurable viremia (P= 0.000016). The HBV DNA levels in serum increased up to 10e9 copies/ml and the viremia continued for more than 2 months. Nucleotide sequence analysis showed that revertant viruses with nucleotide substitutions revert the stop codon to amino acids. Interestingly, not only an original wild type revertant (TAA to CAA), but a novel virus with a nucleotide sequence of AAA instead of CAA also emerged.

Conclusions:Complementation of the HBx protein with hydrodynamic injection rescued the HBx deficient virus infection to the human hepatocyte chimeric mice, and resulted in the emergence of the revertant virus. Our results suggest that the HBx protein is necessary in active replication of the virus in vivo.

Final ID: 818

Reactive oxygen species induce liver fibrosis in uPA/SCID mice with human hepatocytes infected with specific hepatitis B virus genotypes

M. Sugiyama; [1]; *Y. Tanaka;* [1]; *I. Maruyama;* [2]; *T. Shimada;* [2]; *S. Takahashi;* [4]; *T. Shirai;* [4]; *K. Hino;* [3]; *I. Sakaida;* [3]; *M. Mizokami;* [1];

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Abstract Body: Background: Hepatitis B virus (HBV) genotypes/subgenotypes indicate different clinical features, i.e. genotype C is associated with hepatocellular carcinoma, and Bj with precore mutation (PCm) is associated with fulminant hepatitis. We previously showed the difference of each genotype in vitro and partially in vivo. **Aim**: Our purpose of this study is to investigate the difference of viral replication, protein production and liver damage among HBV genotypes in vivo. **Methods**: Sera of six HBV carriers [three each for Bj_wild-type (from acute HB patients) and Bj_PCm (fulminant HB patients)] were prepared, and the other sera were obtained from pre-infected mice which were inoculated with culture medium produced by transfection with plasmids (3 strains each for Ae, Ce, Bj_wild-type and Bj_PCm) carrying HBV genome in Huh7 cells. These 18 sera were inoculated into uPA/SCID mice harboring human hepatocytes (chimeric mice). HBV DNA in mouse serum was measured by real-time detection PCR weekly, and alpha-smooth muscle actin (α-SMA)and 8-OHdG were detected by immunostaining 6 month post-infection. Reactive oxygen species (ROS) was detected by *in situ* hybridization with dihydroethidium. **Results**: Chimeric mice inoculated with HBV/C or Bj_PCm for 6 months showed that human hepatocytes had somewhat ground-glass appearance and fibrosis, while chimeric mice inoculated with HBV/Ae or Bj_wild-type were not induced. Immunostaing analysis showed strong staining of α-SMA around fibrosis. ROS and 8-OHdG were highly expressed in HBV/C and Bj_PC mice, and ALT and TGF-beta1 production of fibrosis group were higher than that of non-fibrosis group (*P*). The HBV-DNA level in sera was the highest in mice infected with HBV/C compared to those with HBV/A and HBV/B (10^9, 10^7 and 10^4 log copies/ml levels, respectively, *P*) during 6–8 weeks post-inoculation. HB core-related antigen excretion had a similar trend to replication, whereas secretion of HBsAg was more pronounced for HBV/A followed by HBV/C and much lesser for HBV/B. Introduction of precore stop-codon mutation in the HBV/B caused significant increase in viral replication, antigens expression, and the histopatological picture similar to HBV/C. **Conclusion**: Using humanized in vivo model, we demonstrate that different HBV genotypes and even particular mutation resulted in different virological and histopathological outcomes of infection, indicating that distinct HBV variants may be directly cytopathic in immunosuppressive conditions. This would represent a novel mouse model for human liver fibrosis associated with ROS production leading to the activation of hepatic stellate cells by infection.

Final ID: 949

Serine palmitoyltransferase inhibitor suppresses hepatitis B virus replication

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Abstract Body: Background: 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 can suppress HBV replication.

Aim: To investigate the anti-HBV effect of myriocin against intact HBV using Huh7 cells transfected with HBV (in vitro) and uPA/SCID mice with the liver replaced by human hepatocytes (chimeric mice) with HBV (in vivo).

Method: Huh7 cells were transfected with plasmids carrying 1.24-fold the HBV genome of genotype C which is the most common in Asia. Myriocin was added in the medium of Huh7 cells at a final concentration of 0.1 to 20 μM. After 72h incubation, culture supernatants were collected and HBV-DNA was quantified by real-time detection PCR to determine the 50% inhibitory concentration (IC50) of myriocin. These culture media were also injected into chimeric mice. Myriocin or pegylated interferon (PEG-IFN) and the combination were treated in chimeric mice (n=9) for a week, and HBV-DNA in mice serum was measured by real-time PCR. PEG-IFN was subcutaneously injected at 30 μg/kg (3 times injection per week). The amount of myriocin intraperitoneally injected was adjusted to be 1 mg/kg.

Result: We succeed in reducing the HBV DNA levels in culture supernatants of Huh7 cells. As a result, IC50 value of myriocin was about 5 μM. Although we administered myriocin into HBV-infected chimeric mice and it was not effective enough to reduce HBV-DNA levels, PEG-IFN reduced the HBV levels in serum to 1/10 of the levels prior to 8 days treatment. Interestingly, myriocin with PEG-IFN reduced the HBV levels to less than 1/1000 of the control levels. Concurrently, we monitored the concentration of human albumin and found slightly reductions only in the combined treatment group, but not reaching to significance.

Conclusion: (Myriocin could inhibit HBV replication in Huh7 cells. Although myriocin monotherapy was not effective, combined treatment with PEG-IFN cooperatively and synergistically inhibits the replication and proliferation of HBV in a chimeric mouse model of humanized liver.) Our results suggest that SPT may be an effective target of drugs designed to inhibit HBV replication, and that SPT inhibitors such as myriocin are good candidate on which to base the development of new anti-HBV drugs.

Final ID: 1226

Evaluation of Pegylated Interferon plus Ribavirin-induced Selection and prevalence of Amino acid 70Q in Core of HCV-1b

F. Kurbanov; [1]; *Y. Tanaka;* [1]; *K. Matsuura;* [1]; *F. Sugauchi;* [1]; *T. Sakamoto;* [1]; *I. Hasegawa;* [2]; *T. Ohno;* [2]; *M. Mizokami;* [1];

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Abstract Body: Background: Pegylated interferon (PEG-IFN) plus ribavirin (RBV) therapy for hepatitis C virus (HCV) leads to sustained virological response (SVR) in approximately half of genotype 1b-infected patients. Null response (NR) or robust reduction with following relapse (REL), are two principal forms of the failure. Recent studies indicated that amino acid pattern in HCV core(70Q and/or 91M) or interferon sensitivity determining region (ISDR) in NS5A are strongly associated with poor virological response.

Aim: To investigate whether treatment-induced selection occurs in the HCV genetic regions previously associated with sensitivity to interferon.

Methods: Sixty Japanese patients with chronic HCV infection (M:F 37:23, age 53±12, ALT 63.4±39.1 U/L, PLT 16.1±6.0x104/μl, HCV RNA 2311±1600 KIU/mL) were treated with PEG-IFN-α2b (1.5 μg/kg/week) plus RBV (600-1000 mg/day) for 48 weeks. Sera were collected at baseline (BL), on-treatment (weeks 4-48) and 24-48 weeks after therapy cessation. Part of Core and ISDR were directly sequenced. Treatment with different regimens was modeled on five uPA/SCID mice carrying human hepatocytes (chimeric mice).

Results: In studied cohort, SVR, NR and REL established in 26(43.3%), 15(25%) and 16(26.7%). Remaining 3(5%) dropped. Compared at BL, prevalence of the 70Q tended to be higher in the NR and REL than in SVR group (36% and 29% vs 19%). In NR group the prevalence at BL, on-, and after treatment was 36%, 50% and 74%, respectively, i.e, after treatment, the 70Q prevalence in NR was significantly higher than in SVR before treatment ($p=0.009$). In two of the four NR cases with 70R, the interferon “sensitive” core coincided with “resistant” ISDR, and in the remaining two cases had documented reduction of the received medication. Serum specimen from one NR patient (with 70R before, on and after treatment) was used for infection of 2 chimeric mice. In both mice, substitution 70Q emerged after 3 injections of PEG-IFN-α2a (3.0 μg/kg) plus RBV (50 mg/kg). No changes observed in any of three mice infected with SVR strain. In addition, prevalence of the 70Q investigated on 3315 GenBank entries, was significantly higher in subtype 1b (54.2%) than 1a, 2, 3 and 4 (1.9%, 0%, 8.6% and 13.4%, respectively). Of the 1b strains, the prevalence was significantly higher among strains published from Europe and America than those from Asia (59%, 63% vs 44%, $p<0.00001$). **Conclusions:** The results indicate that 70Q might be an important viral factor involved in PEG-IFN-induced selection of the resistant strains. Further studies are required to investigate mechanisms of this involvement and to confirm the geographic differences in 70Q prevalence.

Final ID: 1906

Telaprevir treatment in combination with interferon-alpha to a human hepatocyte chimeric mouse with hepatitis C virus infection

E. Iwao; [1]; *M. Imamura;* [2, 3]; *N. Hiraga;* [2, 3]; *T. Kimura;* [2, 3]; *D. Miki;* [2, 3]; *T. Hatakeyama;* [2, 3]; *F. Mitsui;* [2, 3]; *M. Tsuge;* [2, 3]; *N. Kamiya;* [1]; *S. Takahashi;* [2, 3]; *K. Chayama;* [2, 3];

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Abstract Body: Purpose: Clinical studies to achieve eradication of hepatitis C virus (HCV) infection using a novel NS3/4A protease inhibitor, telaprevir, in combination with peg-interferon (IFN) or peg-IFN and ribavirin are now progressing. However, there has been no report about the combination study of telaprevir using an animal model. We previously reported that telaprevir (Kamiya, 57th AASLD meeting, 2006) as well as IFN-alpha (Hiraga, FEBS Letters, 2007) could inhibit HCV replication in a HCV-infected human hepatocyte chimeric mouse. In this study, we examined the efficacy of combination of telaprevir and IFN-alpha using the mouse model.

Methods: The homozygous urokinase plasminogen activator-SCID mice transplanted with human hepatocytes (>70% of the liver) were used. Twenty-one mice were inoculated with genotype 1b HCV-infected patient serum and achieved successful HCV infection. The mice were daily treated with IFN-alpha alone (1500 IU/g, s.c., once a day), telaprevir alone (200 mg/kg, p.o., bid) or both combined for 14 days (Period 1; P1) and following another 14 days (Period 2; P2). Animals were divided into following six groups: A; IFN-alpha in P1 and P2 (n=4), B; telaprevir in P1 and P2 (n=4), C; combination in P1 and P2 (n=4), D; IFN-alpha in P1 and telaprevir in P2 (n=3), E; telaprevir in P1 and IFN-alpha in P2 (n=3), and F; combination in P1 and telaprevir in P2 (n=3).

Results: In P1, IFN-alpha, telaprevir and combination led to 1.49, 1.91 and 2.37 log10 decreases in serum HCV RNA levels after 3 days, respectively, and 1.80, 1.88 and 3.37 log10 decreases after 14 days, respectively, indicating additive effects of both agents. In P2, most mice showed plateau or continuing decline of viral response. However, increase of HCV RNA levels in P2 was observed in two mice of group B (telaprevir mono) and a mouse of group C (combo) among groups A to C, V36A mutation was detected in the NS3 protease region of the breakthrough HCV from a mouse of group B by direct sequence analysis. Among groups D to F, HCV RNA levels in two mice of group F (combo followed by telaprevir mono) increased more than 1 log10 in P2. This finding might indicate the importance of IFN-alpha to inhibit growth of telaprevir-resistant variants in vivo, and sequencing study is now on going.

Conclusion: The additive effect observed in this study was similar to the clinical results of a small phase Ib trial of telaprevir in combination with peg-IFN (Forestier, Hepatology, 2007). Under an enough selective pressure, emergence of drug resistant variants could be observed in this small animal model.